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COMPARISON OF PEANUT MEAL, RAPESEED MEAL AND SOYBEAN MEAL AS PROTEIN SUPPLEMENTS FOR PIGS AND LABORATORY RATS

by

Ⓒ ETIM JONATHAN OROK

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
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IN ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA
FALL, 1973

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Comparison of peanut meal, rapeseed meal and soybean meal as protein supplements for pigs and laboratory rats", submitted by Etim Jonathan Orok, B.Sc., M.Sc., in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Nutrition.

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ABSTRACT

Soybean, peanut (groundnut) and rapeseed meals are important supplements in animal nutrition. Increased knowledge of their relative intrinsic worths would facilitate their optimum utilization in feed formulation and possibly suggest ways of improving their qualities. Using uncastrated male and female Sprague-Dawley rats, The University of Alberta strain, and Pietrain- and crossbred-sired barrows and gilts as the test species, studies were conducted using a control diet supplemented with solvent-extracted soybean meal (SBM) to compare prepress solvent-extracted rapeseed meal (RSM) from Brassica campestris cultivar Span seed, with solvent-extracted peanut meal (PNM) when these protein sources were fed as sole protein supplements or in various combinations, with or without added lysine. Evaluation criteria were food intake, gain, efficiency of food conversion (EFC) and carcass traits in both species; energy and nitrogen (N) utilization, postprandial plasma urea and amino acid concentrations in pigs only and rat thyroid weights and histology.

Rats and pigs responded differently to RSM- and PNM-supplementation of diets. When food intake, gain and EFC were employed as the criteria of comparison, PNM proved to be as good a protein supplement as SBM for rats; whereas with pigs, SBM was the best protein supplement, followed by RSM, followed by PNM. With rats, increasing substitution of RSM for PNM considerably reduced food intake, gain and EFC. With pigs, a systematic, although not always statistically significant, improvement in these traits was generally associated with increasing substitution of RSM for PNM. Rats did not respond positively to the addition of lysine to the PNM-supplemented diet in food intake, gain and EFC; while pigs did, although not statistically significantly. In these three traits, uncastrated male rats surpassed ($P \leq 0.01$) female rats. Only in EFC did gilts excel ($P \leq 0.05$) barrows in both replicates. In the other two traits, the trend of response between the two sexes varied between replicates. There were no significant breed differences in feed intake, gain and EFC, although Pietrain-sired pigs tended to be slightly inferior to crossbred-sired counterparts.

No significant treatment, sex, or breed differences were observed in energy digestibility or in metabolizable energy. In N retention, gilts were superior ($P \leq 0.05$) to barrows and Pietrain-sired pigs were superior ($P \leq 0.05$) to crossbred-sired pigs, consistent with the leaner ($P \leq 0.05$) carcasses of gilts and Pietrain pigs.

Sex, but not dietary treatments, influenced the thyroid weight of rats, with female thyroids being larger ($P \leq 0.01$) than those of males. Based on histopathology of the thyroids, RSM, SBM and PNM were goitrogenic in that order.

Under conditions of unstandardized feed intake, plasma urea concentration proved to be as valid a criterion of protein evaluation as any other when comparisons were based on 0 - 7 h percentage increase in postprandial urea output/g N ingested on the morning of blood sampling. Postprandial plasma amino acid concentrations closely reflected dietary amino acid levels. A high and early plasma lysine peak followed by a comparatively rapid decrease in postprandial plasma lysine concentrations of pigs fed RSM-supplemented diets suggested an inhibition of transient storage of lysine in the muscle of pigs fed RSM-supplemented diets.

It was concluded that the various evaluation criteria employed in these studies are useful in evaluating dietary protein supplements, especially when used together. The relative ranking of the protein supplements depends, however, on the test species.

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INTRODUCTION

Rapeseed as a commercial crop was introduced into Western Canada in 1942 as a war measure to supply oil for lubrication of marine engines (Bowland, 1965). Today, Canada exports more rapeseed than the rest of the world together. In 1969, for example, Canada produced 606,000 metric tons of rapeseed compared with 252,000 metric tons in 1960: a 240% increase in one decade (Wijsman, 1970). The production for 1970, 1971, and 1972 respectively, were 1.64, 2.15 and 1.30 million metric tons (Statistics Canada, 1971, 1973). Rapeseed acreage in 1973 decreased by 4% from that seeded in 1972. Although production has decreased in the last two years, the overall growth of the rapeseed industry in Western Canada has been phenomenal. This growth has come about through research and the application of research findings to the needs of the industry. If the past growth of the Canadian rapeseed industry was "phenomenal", the future is probably even brighter, because the facts and tools are now available for plant breeders to make substantial genetic intervention in the evolutionary course of rapeseed production in Canada.

Such intervention among other things, aims at improving the chemical composition of rapeseed so that its by-products such as rapeseed meal (RSM) can meet the changing demands of their users on a competitive basis with similar or alternative products entering the world market. As new varieties of rapeseed become available, therefore, they must be processed and the by-products evaluated from time to time so that information may be made available to plant breeders for guidance in future breeding programs.

A by-product of rapeseed industry of interest to animal nutritionists is RSM. Almost all Canadian studies evaluating the nutritive value of RSM have compared it with soybean meal (SBM) and occasionally with linseed meal; whereas, SBM is only one of the oilseed meals with which Canadian RSM competes in the world market.

In the present study, prepress solvent-extracted RSM from the 1971 crop of a recently evolved and widely cultivated rapeseed variety "Span" was evaluated in comparison with peanut meal (PNM) and SBM, using starting-growing pigs and weanling rats. The evaluation techniques and the response criteria adopted were aimed at reflecting the current concern of Canadians for efficient production of lean carcasses and at identifying the limiting factors inherent in each of the protein supplements tested.

LITERATURE REVIEW

THE GROWING IMPORTANCE OF OILSEED MEALS IN WORLD FOOD ECONOMY

Oilseed meals are by-products of the vegetable oil industry. The past three decades have witnessed a substantial increase in oilseed meal production (FAO, 1972). This increase is a recent phenomenon attributable to the growing dietary importance of vegetable oils. Until this century, the main fats eaten by man were animal fats. Vegetable oils served mostly as illuminants. In north-western Europe, for example, 'colza' or rape oil was fuel for lamps (Pyke, 1970). Many factors have contributed to the displacement of animal fats by vegetable fats (Call, 1970; Pyke, 1970 and Rice, 1970). Some of these factors are technological change, food preferences and the dynamic nature of food consumption, internal and external price support policies, bilateral trade agreements, population growth, and the fact that compared with animal fats, most vegetable fats are lower in saturated fatty acids and the current suggested association of high intakes of saturated fats with altherosclerotic diseases (Kannel, 1971).

As the emphasis on human leaness through dietary manipulation extends to the feeding of the animals that man consumes, it is conceivable (Rice, 1970) that, in time, the demand and prices for oilseed meal, the protein by-products of vegetable oil industry, may become so high that oilseeds are selected and grown for their meal contents, with the vegetable oil as by-product. The relative price situation justifying this alternative appears to have been reached during the past year, when protein supplements for animal feeds became so scarce and expensive that meat prices soared. Given the current underlying factors alone, it is
② evident that the horizon is decidedly in favour of increased oilseed

meal production. What is not so evident is the role that the individual oilseeds or oilseed meals will play in the future world food economy. However, to the extent that "science and technology underpin world economies, their security, public health and food supply" (Handler, 1971), only those oilseed meals that continually receive adequate research attention will survive the economic realities of the future and continue to contribute to human welfare.

SOYBEAN, PEANUT AND RAPESEED AS PROTEIN SUPPLEMENTS IN ANIMAL FEEDS

Soybean (*Glycine max*), peanut (*Arachis hypogaea*) and rapeseed (*Brassica napus* and *Brassica campestris*) all have long histories as cultivated crops. Soybean originated from China where it was recognized as an essential food crop before 2,838 BC, in which year it was first described (Ferrée, 1929). The origin of oilseed rape is uncertain, although mention of it in ancient Indian Sanskrit writings of 2,000 to 1,500 BC is reported (Downey, 1965). Peanuts existed as early as 950 BC and were found first in South America (Woodroof, 1966). Soybean was introduced into North America in 1910 (Ferrée, 1929), peanut, 1865 (Woodroof, 1966) and rapeseed, 1942 (Bowland, 1965). Before then, comparatively little was written about them and each has made its recorded history in North America. It is in North America and to a lesser extent, Europe and Asia, that their meal by-products, soybean meal (SBM), peanut meal (PNM) and rapeseed meal (RSM) are continually being scrutinized for inherent qualities and demerits with a view to systematically improving them and making optimum nutritional use of them.

SBM and PNM have higher crude protein (N x 6.25) and digestible or metabolizable energy than RSM; but in amino acid composition, RSM compares more favourably with SBM than does PNM (NAS - NRC, CDA, 1969). RSM is lower in lysine but higher in methionine than SBM. RSM also compares favourably with SBM in minerals and vitamins, being higher in phosphorus, selenium, magnesium, choline and niacin (Clandinin *et al.*, 1972). PNM is more deficient in lysine and methionine, but higher in arginine than the other two meals. These differences in nutrient composition provide potentialities for mutual supplementation in diet formulations. The magnitude of the differences in amino acids, particularly ④ lysine, may be modified by processing, depending on the degree of heating during processing.

It has been suggested (Jones, 1969) that for protein concentrates such as SBM, PNM and RSM, their analysis for lysine would be a better guide to their biological value than an analysis for crude protein. Much as this is true, experience indicates that in the evaluation of protein supplements, some consideration should be given to growth inhibitors, such as goitrogens in RSM (Bell, 1965; Kennedy and Purves, 1941; Viehover *et al.*, 1920), aflotoxin in PNM (Barber *et al.*, 1968; Dutchie *et al.*, 1968), and antitrypsin in SBM and PNM (Anantharaman and Carpenter, 1969; Bielorai, 1969; Borchers *et al.*, 1947). In Canada, ⑤ this aspect is considered in the use of RSM in poultry and animal feeding; and for each class of animals, safety limits are set from time to time, based on research findings.

Today, it is evident from production statistics (FAO, 1972) that soybean, peanut and rapeseed in that order, rank first, third and fifth respectively as world oilseeds. Nationally and internationally, the future relative status of each will depend on the net impact of the co-ordinated interdisciplinary research effort that each receives.

EVALUATION OF PROTEINS

Proteins have provided problems for investigation for centuries (Vickery and Schmidt, 1931). Today, the growing international concern over the problem of meeting the world dietary protein needs in the face of an expanding world population (Anon., 1968) testifies to the existence of a continued need for investigations on proteins and calls for precise and empirical methods of evaluating protein quality. Arising from this need, NAS - NRC (1963) reviewed and outlined procedures of evaluating protein quality. This review was comprehensive in 1963, but now shows gaps and leaves room for new approaches. Omitted in the review, for example, is the use of plasma urea concentration as a predictor of protein quality. Also not considered were carcass criteria such as the use of record of performance (ROP) score and yield index (carcass grade), which have proved to be potent evaluation criteria employed in the Canadian pig industry. Among the many techniques included in the review are crude protein, animal growth, nitrogen (N) balance index, amino acid analyses, chemical score and plasma amino acid concentrations. Those wishing to evaluate protein can therefore select and possibly supplement these procedures or combinations thereof according to circumstances and need. As will be seen later, each evaluation technique has advantages and disadvantages; but in combination, they can be used advantageously to answer specific questions on dietary protein quality.

CRUDE PROTEIN AS INDEX OF PROTEIN QUALITY

The use of Kjeldahl or Dumas N analysis to estimate the protein content of foodstuffs rest on the premise that all proteins contain 16% N and that therefore, the crude protein percentages of N-containing compounds can be obtained by multiplying the N content by 6.25. In fact, not all proteins contain 16% N; and although not used in routine analysis, specific factors have been determined (Jones, 1941) for different proteins and foods. Moreover, some of the N in the N-containing compounds exists as non-amino acid N in various chemical forms such as purines, pyrimidines, vitamins, creatine, creatinine and urea, although the contribution of these to the N economy of monogastric animals is probably very little (NAS - NRC, 1963). Much as it offers a fast method of comparing "crude protein" contents, routine determination of protein as "N x 6.25" instead of on the crucial basis of amino acid content and pattern falls short of accurately portraying protein quality.

ANIMAL WEIGHT GAIN AS RELATED TO PROTEIN QUALITY

Animal weight gain, frequently referred to as growth, a quantitative variable easily measurable by scale, is of practical interest to the animal producer. Gain is substantially responsive to planes of nutrition and is widely used to compare the nutritive value of diets. A major disadvantage of using "growth" as a criterion of feed evaluation is that it does not distinguish the composition of gain which itself is dietary-dependent and can vary widely between animals of equal weight and age.

CARCASS ANALYSES

Carcass analysis is the ultimate indicator of carcass composition. Individual muscle dissection would give an excellent measure of marketable lean, but it is slow and laborious. Moreover, it overlooks that portion of carcass protein occurring outside the muscle. With rats, representative samples of whole carcasses may be analyzed to obtain data for estimating the total content of protein (N x 6.25), fat, moisture and ash. Such analysis, of course, does not indicate how much of the protein comes from inedible portions such as fur. Nevertheless, it provides a more realistic estimate of the quality of gain or growth than mere liveweight. Whole carcass analysis usually is not practicable with pigs, partly because of economic considerations.

Historically, the Canadian pig industry has been alert to trade requirements and has consistently adjusted its grading methods and standards to need. The grading system used in Canada (Hog carcass valuation) is an indication of yield. A value-yield index based on extensive Canadian study[1] (Fredeen _et al_., 1968a, 1968b) was introduced (CDA, 1969) for use in routine grading of commercial hog carcasses.

Value-yield index = 63.78 - 5.5 (backfat) + 0.11 (hot dressed carcass). The model evaluates hog carcass according to its predicted lean content, based on the total backfat thickness taken at the shoulder and loin of the split carcass. Financial reward to the producer is calculated as:

Value-yield index X bid price X hot carcass weight (lb.).

---

[1] Fredeen, H. T.: Cross-Canada hog carcass test (1967). Unpublished data.

Record of performance (ROP) for swine, introduced in 1928, is aimed at assisting breeders of purebred pigs in their search for better breeding stock. It provides measurements of carcass merit, age to market and feed requirements (Fredeen, 1966). Initially, it was geared to help breeders select lean hogs. This was compatible with the recognized commercial grades operating at that time. With the shift of emphasis in the early sixties to leaner carcasses, ROP index was amended (Fredeen, 1966). The most recent revision based on new evidence is designed to give a predicted yield of wholesale cuts and is calculated as follows:

$$\text{ROP}^{1} = 51.68 - 3.234\,(A) + 1.0381\,(B) + 0.485\,(C) + 11.76\,(D) \pm E$$

where A = Total backfat (sum of shoulder back and loin, in.)

B = Loin area (area of loin at 14th rib, sq. in.)

C = Ham/carcass (%)

D = Area lean of ham/ham weight (sq. in./lb.)

E = Sex correction factor (+ 1.1% for barrows; - 1.1% for gilts).

At the present time, this index is too elaborate for use in routine grading of market hogs, but is useful in assessing pig carcasses in nutritional research studies.

N BALANCE AND RETENTION AND RELATIONSHIP TO PROTEIN NUTRITURE

The Kjeldahl method of N analysis is simple, fast and reproducible and can be used to measure the N content of ingested feed and of feces and urine in N balance studies. The minimum period for collecting feces

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[1] Source: T. A. Omole, 1973 Ph. D. thesis entitled "Copper supplementation of swine diets as influenced by protein source and trace mineral supplements"; pp. 46 - 47; Dept. Anim. Sci., University of Alberta, Edmonton, Alberta, Canada.

and urine and for recording feed intake in N balance studies is three days (NAS - NRC, 1963), following a suitable adaptation period, usually 2 - 3 days. The difference between an animal's N intake and N output is its N balance. This value may be negative, zero or positive. When zero, an animal is said to be in balance. Mature animals normally show zero balance. Zero balance can be attained at various levels of N intake (FAO, 1965; Rodwell, 1969), due to the phenomenon of adaptation to N intake, of which individual animals are capable.

N balance expressed as a percentage of N intake gives the percentage of absorbed N retained by an animal. To the extent that "6.25" or more specific factors can be used to convert N to protein, N balance studies can portray protein nutriture (absolute and percentage digestibilities as well as biological values (BV)). It is always essential to specify the level at which the N or protein was fed and to ensure good management and random distribution among treatments of those factors such as age, sex and breed of animals all of which affect the results obtained. This implies, among other things, that nutrients other than those tested are adequate in the diet.

N balance is a more realistic measure of the gain in lean than the "growth" criterion of protein evaluation and because N balance can be determined in vivo, it has advantage over some alternative procedures such as carcass analyses, which entail slaughtering the animals. In the case of adult animals in particular, N balance has the disadvantage that it does not necessarily represent protein adequacy, since N equilibrium can be attained at various levels of N intake. There are also other inherent weaknesses of N balance studies, namely: feed wastage and contamination of urine with feed and feces which are common.

Moreover, quantitative collection of both feces and urine is difficult; and apart from fecal and urinary losses, there are other sources of N loss such as sweat, gaseous products of digestion and desquamation of the skin and hair growth, especially in long term studies.

AMINO ACID COMPOSITION AND PROTEIN QUALITY

For many years after the discovery of proteins, the prevalent view was that protein nutrition was essentially N nutrition. Consequently, diets were formulated and minimum dietary requirements set on the basis of proteins, until 1897, when Rubner recognized that proteins of varying origins were not of the same value nutritionally and that therefore there was no protein minimum but as many minima as there were proteins (Block and Mitchell, 1946). For those interested in the revolutionary changes that have shifted emphasis from protein to amino acid nutrition, the review thus cited is significant and makes interesting reading. It covers among other things, the emergence of the concept of amino acids, the classical use of purified diets by Osborne and Mendel in the early part of this century to study amino acid supplementation, the distinction in the thirties by Rose between essential and nonessential amino acids, the techniques of amino acid analyses and the difficulties associated with each, the attempts to correlate the nutritive values of proteins with their amino acid composition, and the nutritionists' confrontation with unresolved problems of amino acid nutrition. Indeed, amino acid nutrition has gone a long way since its inception. Today, some 25 amino acids have been identified, characterized and their metabolic or dietary essentiality established.

But many problems still face the nutritionist. For example, in the hydrolysis of proteins for amino acid analyses, no procedure has yet been found which will completely release all the individual amino acids without loss of some. In acid hydrolysis, all the tryptophan and variable amounts of serine and threonine are destroyed; glutamine and asparagine are deaminated to glutamate and aspartate; glutamic acid undergoes intramolecular dehydration to pyrollidone 5-carboxylic acid, and other amino acids may undergo intramolecular dehydration forming cyclic anhydrides or diketopiperazine (Rodwell, 1969). Microbiological assay overcomes some of these problems and may facilitate amino acid sequence studies. However, it has unique problems of its own as enumerated by Rodwell (1969) and NAS - NRC (1963). But the use of microbiological and chromatographic procedures can mutually supplement each other and provide an independent check of the values obtained by each method.

Chromatographic methods of analysis, however, do not normally distinguish between D and L forms of amino acids. This is a potential shortcoming, as studies of the nutritive values of the optical isomers of the essential amino acids have shown that generally speaking, although there are species differences, only the naturally occurring form (L-) is utilized (Maynard and Loosli, 1969). There are also exceptions such as methionine where both D and L isomers are active for higher animals. Knowledge of the amino acid analyses of a feed does not indicate how much of it is available to the animal; and apart from availability considerations, it is now evident that the absolute content of an individual amino acid is not the only determinant of protein quality. Amino acid patterns and associative effects with other nutrients and dietary ingredients are important.

CHEMICAL SCORE AS AN INDICATION OF PROTEIN QUALITY

For protein synthesis to occur, all needed amino acids must be present at the site of synthesis at the right time (Schaible, 1970). Protein synthesis is limited by the most deficient amino acid and is therefore said to follow the law of the minimum. As noted above, the amino acid pattern of proteins rather than the absolute amount relative to the total N content of the protein is more important. The importance attached to amino acid patterns led Block and Mitchell (1946) to introduce chemical score. Chemical score determination involves relating the essential amino acid composition of a test protein to that of a standard protein, usually whole egg, or to the amino acid requirements of a given species and physiological group, where the requirements are known. The content of each of the essential amino acids of the test protein is expressed as a percentage of the corresponding amino acid of the standard. The lowest percentage is the chemical score. The larger the chemical score, the better the protein. The significance of the values so obtained is species-dependent. The values seem to correlate well with the BV for rats and human beings but not for poultry (McDonald _et al_., 1966).

Chemical scores have two major advantages: first, once the amino acid composition of the test proteins are determined, the chemical score can readily be calculated from the amino acid composition of the standard. Secondly, where supplementation with one limiting amino acid or the other is contemplated, the chemical score is helpful in determining priority. Chemical score has two major disadvantages. It will not reveal the effect of imbalance in pattern which can reduce effective utilization of the limiting amino acids (FAO, 1965); albeit, if the values are compared with BV, any discrepancy thereof could suggest imbalance or reduced availability or both. Chemical score overlooks all but the most limiting amino acid. This is particularly serious in cases such as observed in certain heat-damaged samples of peanuts where, two or more amino acids must be supplemented together in order to elicit growth response (Anantharaman and Carpenter, 1971).

PLASMA AMINO ACID PATTERNS AND PROTEIN NUTRITION

One of the most recent techniques of protein evaluation is that of observing the plasma free amino acid concentrations. This involves feeding to animals, after starvation for 18 h or more, a diet containing a test protein or a mixture of test proteins and measuring the plasma amino acid concentration at suitable intervals, usually 0.5 - 1 h, after feeding. The observed concentrations are subjected to one of many alternative calculations, from which the quality of the ingested protein can be assessed. Five to 6 h overall sampling period is usually sufficient, since most investigators (Denton and Elvehjem, 1954; Myres, 1970; Rérat, 1972) have reported amino acid peaks within 3 h of refeeding.

The technique of plasma free amino acid concentrations is based on the classical view that dietary proteins when ingested are completely degraded to amino acids, in which form they are absorbed by the intestinal mucosa and transported through the portal system to the liver, whence they enter the general circulation to be utilized at various sites according to need. Because of the unique transportation function of plasma in the animal body, it provides a suitable medium for study, since the pattern of substances transported by it at any one time could reflect the state of welfare of the organism studied. Given a suitable bench mark from which feeding is administered and the pattern of the desired substance observed, it could also reflect the dietary contribution of that substance to the animal's welfare.

This idea has prompted many investigators to try to relate plasma amino acid patterns to dietary amino acid profile and to the overall biological response. In so doing, they hope to be able to answer specific questions related to amino acid nutrition, such as identifying the sequence of limiting amino acids, and determining the amino acid requirements. As reviewed (Leathem, 1968), the background to this concept is not new. It dates back to Howell (1906), who reported that the concentration of free amino acids in portal blood increases after a protein meal, a finding which has since been confirmed by several investigators (Folin and Denis, 1912; Periano and Harper, 1963; Porter and Williams, 1963; and Van Slyke and Meyer, 1912). Most investigators have also noted a rise in postprandial amino acid concentrations of systemic blood; although this rise is generally less than that of portal blood.

A relatively low peak in the postprandial amino acid concentration of systemic blood compared with that of portal blood is not surprising, being consistent with the known event of the circulation of nutrients through the body. Portal plasma amino acids for which Dawson and Porter (1962) found the highest activity, represents the amino acids available to the animals unaffected by synthetic activity, other than that which has occurred in the walls of the gut. Systemic plasma amino acids, on the other hand, represent the balance left after uptake by the tissues. In the case of systemic blood, the peak is the consequence of the difference between absorption rate and overall tissue uptake. In studies involving many animals, the use of catheters to sample blood may not be feasible. In such situations, monitoring the changes in the systemic amino acid concentration, by using blood from the anterior vena cava may be preferred to that of the portal system. Provided that the samples are properly collected and handled, and the analytical procedures sufficiently refined, meaningful results can be obtained by studying systemic amino acid concentrations.

Plasma amino acid studies have inherent problems, which make it difficult to establish standard values of postprandial amino acid concentrations for a particular protein or protein mix, bearing in mind the wide range of levels or combinations in which such proteins can be applied in practice. Plasma amino acid concentrations are affected by the rate of stomach emptying, which as reported (Porter and Rolls, 1971) depends on several factors which may be listed: firstly, the nature and level of the protein supplements and the composition of the diet as a whole. Secondly, the physical state of the meal; liquid or finely divided meals, for example, leave the stomach quicker than coarser ones. Thirdly, the emotional state of the animal: fear or excitement in so far as they affect adrenocorticoid hormone output may modify the rate of stomach emptying and the amino acid flux. Fourthly, training: for instance, prior training to eat for short daily periods increases the rate of passage of food, and increasing the amount fed per meal increases the absolute rate of stomach emptying; and fifthly, other dietary constituents. A case in point is the transport of some amino acids, which depends on pyridoxine (vitamin B_6), the absence of which may cause certain amino acid deficiencies in protein supplements high in those amino acids (Guyton, 1969). All these and other factors can conceivably modify to varying degrees the postprandial amino acid patterns, thus making interlaboratory comparisons difficult. Then too, in blood sampling, preparation and storage of plasma samples, and in amino acid analyses, there are many technical pitfalls (Perry and Hansen, 1969), which unless avoided, could lead to errors in quantitative measurements of plasma amino acids.

Most nutritional or biological studies are subject to the same endogenous sources of variation listed above; so that with care and adequate attention to the correct procedure, the plasma amino acid technique, like some of its predecessors, may represent another advance in protein nutrition research.

PLASMA UREA AND PROTEIN QUALITY

Those amino acids which the body is unable to use due either to excessive amounts, defective acid ratios, unavailability or combinations of these factors are deaminated. The ammonia so formed is toxic to mammalian tissue and is excreted as urea (Pfander, 1969). Many investigators Eggum (1970) with rats and pigs, Kumta and Harper (1961) with rats, and Preston _et al_. (1965) with sheep, have reported that blood urea concentration is positively related to protein level and inversely to protein quality. Like plasma free amino acids, blood urea concentration depends also on the interval after a meal (Eggum, 1970; Kumta and Harper, 1961): the concentration usually rising following a meal, and peaking 4 - 5 h after withdrawal of feed. Dietary energy level also affects the blood urea concentration (Preston _et al_., 1965). Kidney status may also influence blood urea levels as renal failure may considerably elevate blood urea concentration (Fonnesbeck and Symons, 1969). Clearly, blood urea concentration is a multi-factorial variable whose precision as a protein evaluation criterion is subject to many underlying factors.

GROWTH INHIBITORS IN RSM, PNM, AND SBM WITH SPECIAL REFERENCE TO GRAVIMETRIC AND HISTOLOGICAL ASSESSMENT OF GOITROGENESIS.

Many toxic substances inhibitory to growth occur in plants; for example, goitrogens in various species of _Brassicae_, carcinogens in oil of safaras, cyanogenetic glucosides in beans and nuts and hemagglutinins in some legumes and castor beans (Oser, 1970). To the extent that the toxic constituents of plants may be lethal, or impair food utilization and depress growth, the protein evaluator must be careful when assigning cause to effects. He must discern the growth and survival attributes that are due truly to protein nutrition, as distinct from the toxic effects arising either from the intrinsic nature of the protein or from the presence of concomitant substances such as goitrogens (Oser, 1970).

RSM (Astwood _et al_., 1949a), PNM and SBM (McCarrison, 1933) all contain growth inhibitors. SBM and PNM contain antitryptic factors which can be destroyed through adequate heat processing (Anantharaman and Carpenter, 1969). RSM on the other hand contains glucosinolates, the hydrolytic products of which (isothiocyanates, oxazolidinethiones and nitriles) are toxic to animals. The heat remedy applicable to SBM and PNM has not been as successful with RSM, since heat intensity sufficient to destroy the glucosinolates could also denature the protein of RSM (Josefsson, 1970). The usual procedure in processing is to inactivate the myrosinase. The glucosinolate content varies considerably between species of rapeseed as does also that of erucic acid, a constituent of rapeseed oil, which is considered to be undesirable. It is known that both the glucosinolate and the erucic acid contents of rapeseed are genetically determined (Downey, 1965). Effort is therefore currently directed at lowering the levels of these constituents through breeding.

Despite considerable genetic gains in recent years, toxicity from goitrogens remains a potential hazard of RSM, except where caution is exercised to limit dietary level of RSM to the recommended optimum, ⑨ (RAC, 1972). Studies involving higher dietary levels of RSM should therefore include, for example, an assessment of the extent of goitrogenesis. In cases where toxity is feared, such study could be undertaken with the cooperation of a toxicologist.

Thyroid enlargement and histopathological changes in thyroid tissue have long been associated with diets containing goitrogens (Hercus and Purves, 1936). Consequently, the extent of these changes have become diagnostic tools for goitrogenesis; and enlarged thyroid glands and histopathological changes in the thyroid gland of animals fed diets adequate in minerals and vitamins (Srinivasan *et al.*, 1957) could signify the presence of high levels of goitrogens in the diet. Such an approach is frequently used with rats and poultry, less so with pigs, due to the difficulty of removing thyroid glands from pig carcasses in commercial packing plants. The gravimetric approach is therefore considerably limited by the relative inaccessibility of the thyroid gland. The same is true of the histological technique, which is further hampered by the fact that it often requires interdisciplinary cooperation which, much as it is desirable, is sometimes difficult to effect in practice.

EFFICIENCY OF FEED CONVERSION (EFC)

This is the feed per unit gain or its reciprocal. It is often used in animal studies to measure the biological response, the quality of diet or the management skill. In all three aspects of use, the concept is of limited value for many reasons. First, the feed intake is often expressed on as fed basis whereas in practice, the moisture content of the feeds compared may vary widely. Secondly, unless the dietary energy value is specified, it is difficult to assign meaningful interpretation to EFC's. Although in practice most investigators specify the energy concentration of the diets fed, interlaboratory comparisons are sometimes difficult due to the investigators' freedom to choose dietary energy levels. Conventional EFC has also been criticized for not taking into consideration the proportion of feed intake used by the animal for maintenance (Meyer and Garrett, 1969) and for representing a spurious statistical correlation of data derived from weight measurements (Tanner, 1949), where, in fact, multiple regression techniques would be most appropriate (Weil, 1962). In spite of these cogent criticisms, feed: gain comparisons still mark the nutrition literature. However, as the animal industry progressively introduces more refined measures of defining quality meat production, the feed: gain technique will become increasingly obsolete.

THE GROWING NEED FOR EFFICIENT PRODUCTION

Intensive animal production, whereby large numbers of animals are confined together and forced to gain rapidly imposes sociological, psychological and other forms of stress that are apt to affect animal performance and nutrient requirements, thus making nutrition a critical factor in production (Church _et al._, 1972). Under extensive management, with relatively fewer animals per unit of operation, a slight reduction in feed conversion, or a slightly higher feed cost may not seem important. To a large intensive producer, however, small changes in these factors can make tremendous differences in the operating expenses and profit.

An analogous situation occurs in plant production, where an increasing cost - price squeeze has led to the use of more fertilizers and cultural and management practices which, while increasing yields per hectare, sometimes result in the production of plant tissues of lower nutritive value or of marked toxicity (Church _et al._, 1972). These considerations together with the growing effort of plant breeders in the production of new plant varieties make it desirable that feedstuffs be periodically evaluated and compared with alternative feedstuffs to establish their relative nutritive status for the benefit of their producers and buyers.

EXPERIMENTAL

OBJECTIVES

The objectives of these studies were:

(1) To assess the nutritive value of prepress solvent-extracted Span RSM as a sole protein supplement or in various combinations with solvent-extracted PNM in comparison with solvent-extracted SBM using pigs of different breeding groups and Sprague-Dawley rats as the test species.

(2) To assess the availability of the lysine in RSM in comparison with PNM and SBM.

(3) To test the relative suitability of postprandial plasma urea and plasma amino acid concentrations as criteria to evaluate protein quality, especially in relationship to gain and carcass composition, which are the practical criteria of performance.

(4) To assess the suitability of solvent-extracted PNM as a sole protein supplement for finishing pigs in comparison with SBM when the meals were fed to pigs of varied nutritional background.

MATERIALS AND METHODS

Pig Experiment, Part 1: Starting Phase.

(a) Animals and Diets

Sixty-four pigs farrowed at The University of Alberta Swine Research Unit were used in this study. Their pre-weaning management followed the standard practice for Alberta as outlined by Bowland and Berg (1967). Weaned pigs before the commencement of the experiment had ad libitum access to creep feed and water while awaiting allotment to experimental diets. In June and September, 1972, respectively, 32 pigs equalized as to sex in each instance were allotted on the basis of equalized weight groups to 8 dietary treatments (Table 1), giving two replicates in time. Replicate 1 consisted of crossbred pigs, half of which (8 males, 8 females) were sired by a Pietrain boar and the rest by boars derived from three- or four-way crosses of Hampshire, Landrace, Lacombe and Yorkshire breeds. They were allotted at an average age of 34 days and an average weight of 5.5 (range: 2.9 - 8.6) kg. Replicate 2 pigs were crossbreds as in Replicate 1, but none were sired by a Pietrain boar. They averaged 36 days of age and 8.6 (range: 6.0 - 11.7) kg at allotment, so were bigger and more uniform in weight than those in Replicate 1. While on test, the pigs were fed individually "to appetite" (Plank and Berg, 1963) by giving them 1 h access to feed three times daily at approximately 8 a.m., 12 noon and 3:30 p.m. When not exposed to feed, they were in groups of four in pens each of which was installed with an automatic drinker and contained pigs fed the same diet.

The diets were formulated to contain approximately 3,400 kcal/kg, 18% (N x 6.25) crude protein, and 0.7% each of calcium and phosphorus, thus giving a 1:1 calcium - phosphorus ratio. Supplemental L-lysine -HCl was added to Treatment 4 containing PNM as the major protein supplement to raise the dietary lysine percentage to 0.76, compared with 0.65 (Table 2) in the PNM-supplemented Diet 3, which had no added lysine. The PNM used in this study was solvent-extracted and purchased from Virginia, U.S.A. The solvent-extracted SBM and the RSM

TABLE 1: FORMULATION AND COMPOSITION OF DIETS[1]

Diet number Supplemental protein source(s)[Δ] %	1 SBM(100)	2 PNM(50) SBM(50)	3 PNM(100)	4 PNM(100) +lysine	5 PNM(75) RSM(25)	6 PNM(50) RSM(50)	7 PNM(25) RSM(75)	8 RSM(100)	9 PNM(50) SBM(50) +lysine	10 RSM(100) +lysine
Ingredients %										
Wheat (ground)	50.0	50.0	50.0	50.0	53.7	52.7	59.8	62.5	50.0	62.5
Barley (ground)	25.3	25.7	26.0	26.0	20.4	19.0	10.3	5.0	25.7	5.0
Stabilized tallow	2.4	2.0	2.0	2.0	2.0	2.7	3.0	3.0	2.0	3.0
Soybean meal (48.5%)	18.0	9.0	----	----	----	----	----	----	9.0	----
Peanut meal (50.0%)	----	8.7	17.4	17.4	13.1	8.7	4.4	----	8.7	----
Rapeseed meal (35.4%)*	----	----	----	----	6.3	12.6	19.0	25.2	----	25.2
Iodized salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Calcium phosphate	1.4	1.8	1.8	1.8	1.7	1.6	1.5	1.4	1.8	1.4
Ground limestone	1.0	0.9	0.9	0.9	0.9	0.8	0.9	1.0	0.9	1.0
Mineral-vitamin premix**	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
L-lysine monohydrochloride (98%)	----	----	----	0.18	----	----	----	----	0.09	0.18
	100.0	100.0	100.0	100.18	100.0	100.0	100.0	100.0	100.09	100.18
Composition										
Digestible energy kcal/kg (calculated)	3426	3405	3415	3415	3403	3414	3427	3410	3405	3410
Crude protein % (analysis)♦	18.7	18.7	18.0	18.7	18.3	18.6	18.5	18.2	18.6	18.9
Lysine % (analysis)♦	0.92	0.78	0.65	0.76	0.65	0.74	0.83	0.88	0.87	1.26
Calcium % (calculated)	0.72	0.73	0.72	0.72	0.73	0.70	0.74	0.71	0.73	0.71
Phosphorus % (calculated)	0.71	0.73	0.72	0.72	0.71	0.71	0.70	0.70	0.73	0.70

[1] First eight diets used for starting pigs: all ten diets used for weanling rats.

[Δ] SBM = soybean meal; PNM = peanut meal; RSM = rapeseed meal. Figures in brackets indicate percentage contributions to supplemental protein. Hereafter, these diets are referred to by number, supplemental protein source(s) and their percentages.

* From Span rapeseed, a mixture of meals from a common source, but processed at three plants.

** The premix provided the following trace minerals: 88 ppm zinc, 76 ppm manganese, 294 ppm iron, 25 ppm copper and 2.8 ppm cobalt. It also provided per 100 kg diet: 440,000 I.U. Vitamin A, 55,000 I.U. Vitamin D, 1,100 I.U. Vitamin E, 1.10 g riboflavin, 2.20 g calcium pantothenate, 4.80 g niacin, 5.50 g choline chloride, 165 mg folic acid and 2 mg Vitamin B_{12}. In addition, 500 g oxytetracycline antibiotic was provided per 100 kg diet.

♦ By analysis of pig diets. Lysine values for rat diets 9 and 10 were estimated from diets 2 and 8 respectively.

were both of Canadian origin. The latter was a random mixture of RSM derived from Span cultivar of Brassica campestris rapeseed grown in one location in the same season, but extracted by the prepress solvent method at three different extraction plants. Span is a low erucic acid type of rapeseed (LEAR) grown extensively in Canada since 1971. The estimated average glucosinolate content of the RSM was 0.65%.

Metabolism Studies

During the 6th to 9th week of the experiment (July 20 - August 23), 3-day energy and N digestibility and retention studies were conducted on all 32 pigs of Replicate 1. These studies were conducted at four periods, each period featuring a pig from each of the 8 dietary treatments, starting with the largest pigs in period 1 and ending with the smallest in period 4. Pigs were fed three times per day at a level equal to 90% of the daily intake during the previous week. The pigs weighed an average of 14.4 (range: 6.1 - 27.4) kg during the 3-day period that feces and urine were collected in metabolism cages. The entire feces and urine were collected from each pig.

Representative samples of the feces were oven-dried[1] (60 C for 72 h) then ground in a Christy and Norris "8 - inch laboratory mill"[2]. Representative samples of the urine were freeze-dried[3] (38 C shelf temperature for 48 h) before gross energy of feces and urine were determined in a Parr Oxygen Bomb Calorimeter[1], with which instrument the energy content of the individual diets was also measured. Kjeldahl crude protein (N x 6.25) determinations of the diets, the dried feces and aliquots of urine, and the dry matter (DM) analyses of the feeds were as described in AOAC (1965). For the Kjeldahl analysis, a commercial "kel-pak"[2] was used to supply the catalyst and the ammonia was collected in a 4% boric acid solution and titrated with standard H_2SO_4.

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[1] Style V31, Despatch Oven Co.; Minneapolis, Minn.; U.S.A.

[2] Christy and Norris Ltd.; Chelmsford, England.

[3] Repp Sublimator, Model SRC 42. Division of Virtis Co.; Inc.; Gardiner, N.Y. 12525, U.S.A.

Blood Sampling and Blood Handling Before Analysis

Blood samples for plasma amino acid and plasma urea analyses were taken during the 9th week of the experiment in Replicate 1 and in the 8th week in Replicate 2 by anterior vena cava puncture (Carle and Dewhirst, 1942) using "17-guage", 3 1/2 - inch needles and 25 ml plastic syringes heparanized[3] just before use. Used syringes and needles were rinsed in physiological saline (0.9% NaCl), then soaked in 70% ethanol (C_2H_5OH), prior to re-use as above.

Due to faster growth and insufficient feed, Replicate 2 pigs were blood - sampled a week earlier than those of Replicate 1. The largest three pigs in each treatment were sampled in Replicate 1 at a liveweight of 11.3 - 45.0 (average 25.6) kg. All 32 pigs of Replicate 2 were blood-sampled. These pigs weighed 17.0 - 40.6 (average 27.8) kg. Six and occasionally seven pigs were bled each day, with each pig from a different treatment. For each pig, the first sample was taken after 24 h fast to provide bench values of plasma amino acid and plasma urea concentrations. Subsequent blood samples were taken from all pigs at 1 , 2 , 3 , 4 , 5 , and 7 h, after refeeding. About 25 ml of blood was withdrawn each time, except in the last two (5 h, 7 h) samples, when approximately 10 ml was taken. Each sample was transferred directly into centrifuge tube(s) to which a drop of heparin had been added. The tubes, thumb-sealed, were gently inverted to mix the blood with the heparin and prevent coagulation. The samples were immediately centrifuged[1] for 10 min. at 2,500 rpm (1.01 g's) approximately and 5 - 10 ml plasma for amino acid analyses were pipetted into plastic centrifuge tubes, sulphosalicylic acid added at about 30 mg/ml plasma and the sample shaken to deproteinise the plasma (Perry and Hanson, 1969). These samples stood in ice water for about 2 h before they were conveyed to the campus for a further 10 min. centrifugation[2] at 14,000 rpm (1.01 g's). The supernatant was stored at -30 C in well-capped, labelled vials until analyses 4 - 6 months later in Replicate 1, and 4 - 5 months later in Replicate 2. For the urea analyses, 2 - 3 ml of undeproteinised plasma samples were retained in storage vials. These were kept in a refrigerator (approximately 0.5 C) at the Swine Research Unit, until the end of each day, when they were taken to the campus for storage at -20C, pending urea analyses.

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[1] Parr Instrument Co.; Moline, Illinois. Temperature changes registered by a Brown Electronik Recorder manufactured by Minneapolis-Honeywell Regulator Company, Philadelphia, Pennsylvania.

[2] Matheson Scientific, East Rutherford, New Jersey. This supplied a mixed catalyst containing HgO, K_2SO_4 and $CuSO_4$.

[3] Sodium heparin anticoagulant. BDH Pharmaceuticals, Toronto, Canada.

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[1] Sorvall Model GLC - I. Ivan Sorval Inc.; Norwalk, Conn. 06852, U.S.A.

[2] Sorvall superspeed RC - 2B automatic refrigerated centrifuge. Ivan Sorvall Inc.; Norwalk, Conn. 06852, U.S.A.

Plasma Urea Analysis

Plasma urea concentration of individual samples was determined by the method of Fawcett and Scott (1960) after 1 - 3 weeks' storage in Replicate 1 and 1 - 2 weeks' storage in Replicate 2.

Amino Acid Analysis and Hydrolysis

The amino acid analyses of pig diets (Table 2) and of blood plasma. (Appendix Tables 1A and 1B) were done with "JLC - 5AH" amino acid analyzer[1]: column specifications as in Appendix i. Feeds were analyzed in duplicate using 1 g samples. Amino acid analysis of blood plasma was on pooled samples in each treatment, 5 ml from each donor for each sampling interval. In Replicate 1, samples were pooled from 3 pigs; in Replicate 2 from 4 pigs. In each instance, 15 ml of pooled plasma was supplied for analyses.

In the hydrolysis prior to amino acid analysis of diets, 25 ml fresh 6N HCl was added to each 1 g feed sample in a 250 ml flat-bottom flask together with about 6 glass beads to minimize bumping. The flasks were then placed on a temperature - controlled reflux apparatus[2] and boiled gently (110 C) for 24 h, before evaporating to dryness at 45 - 50 C with

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[1] Amino Acid Analyzer, Type JLC - 5AH. Japan Electron Optics Co. Ltd.; Tokyo, Japan.

[2] Reflux apparatus. Model 5544. Precision Scientific Co., Chicago, Illinois, U.S.A.

TABLE 2: AMINO ACID COMPOSITION (%) OF DIETS (BY ANALYSIS)

		Starting Diets										Finishing Diets[+]	
Diet numbers		1	2	3	4	5	6	7	8	9*	10*	11	12
Supplemental protein source(s) %	PNM	---	50	100	100	75	50	25	---	50	---	SBM	PNM
	SBM	---	50	---	+	---	---	---	---	50	---		
	RSM	100	---	---	lysine	25	50	75	100	+ lysine	100 + lysine		
Essential amino acids													
Arginine		1.14	1.34	1.53	1.50	1.31	1.26	1.14	0.98	1.34	0.98	0.83	0.99
Histidine		0.48	0.46	0.46	0.43	0.44	0.46	0.48	0.49	0.46	0.49	0.35	0.34
Isoleucine		0.78	0.69	0.66	0.64	0.63	0.66	0.68	0.69	0.69	0.69	0.55	0.51
Leucine		1.36	1.26	1.26	1.21	1.17	1.24	1.26	1.26	1.26	1.26	1.02	0.96
Lysine		0.92	0.78	0.65	0.76	0.65	0.74	0.83	0.88	0.87	1.06	0.66	0.54
Methionine		0.26	0.24	0.24	0.22	0.23	0.27	0.28	0.30	0.24	0.30	0.20	0.20
Phenylalanine		0.93	0.91	0.93	0.90	0.85	0.86	0.86	0.80	0.91	0.80	0.70	0.69
Threonine		0.68	0.60	0.56	0.53	0.55	0.62	0.66	0.70	0.60	0.70	0.50	0.45
Valine		0.88	0.82	0.83	0.79	0.78	0.83	0.86	0.87	0.82	0.87	0.71	0.67
Non-essential amino acids													
Alanine		0.76	0.73	0.74	0.70	0.68	0.73	0.75	0.76	0.73	0.76	0.59	0.57
Aspartic acid		1.62	1.55	1.61	1.55	1.36	1.37	1.27	1.17	1.55	1.17	1.05	1.04
Cystine		0.40	0.36	0.41	0.44	0.40	0.30	0.47	0.47	0.36	0.47	0.39	0.32
Glutamic acid		4.51	4.46	4.74	4.53	4.36	4.44	4.46	4.42	4.46	4.42	3.42	3.45
Glycine		0.79	0.82	0.94	0.90	0.85	0.88	0.87	0.85	0.82	0.85	0.58	0.63
Proline		1.66	1.57	1.51	1.55	1.53	1.56	1.62	1.61	1.57	1.61	1.52	1.46
Serine		0.92	0.89	0.92	0.88	0.83	0.86	0.86	0.84	0.89	0.84	0.67	0.66
Tyrosine		0.48	0.47	0.49	0.47	0.44	0.45	0.44	0.42	0.47	0.42	0.34	0.34

* The amino acid compositions of these two diets included in the rat experiment, were estimated from those of the respective basal diets and the amounts of supplemental lysine.

[+] SBM = soybean meal-supplemented finishing diet; PNM = peanut meal-supplemented finishing diet.

a rotavator[1] equipped with a water immersion heater[2]. After the initial drying, 25 ml deionized water was added and again evaporated to dryness. The final residue was washed into a 100 ml volumetric flask and made to volume with additional deionized water. The resulting mixture was shaken well and filtered.

For the analysis of free amino acids in the plasma, no hydrolysis was required.

Dilution to column capacity and addition of internal standards was as outlined below. For analysis on the columns, 19.2 micrograms of feed protein was used. This was obtained by making a further 4 : 25 dilution from the already 1:100 hydrolysate and injecting 0.8 ml into the columns. The same volume (0.8 ml) of internal standards (amino guanidino propionic acid for the short column and norleucine for the long column) from a 1:25 dilution of a stock solution containing 2.5 micromoles per ml was then added to the final sample solution and the pH adjusted to approximately 2.2, using 6N HCl or saturated NaOH as needed.

Each 15 ml of pooled plasma supplied for analysis was diluted to 25 ml and internal standards added as above. The pH of the final solution was adjusted to approximately 2.5, using 6N HCl or saturated LiOH as appropriate.

Fatty Acid Analysis of Feed Lipids

Feed lipids were extracted with pentane after digestion of the feed with 4N HCl (Vogtmann, 1971). Fatty acid methyl esters were prepared from feed lipids with BF_3 - methanol reagent (Metcalfe and Schmitz, 1961) and measured with Bendix gas chromatograph - 2500[1] (Vogtmann et al., 1973). Results are presented in Appendix Tables2A and 2B but are not discussed as they appeared to be of no assistance in interpretation of data.

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[1] Rotavator - R. Type KRvr 65/45. Rinco Instrument Co. Inc.; Greenville Illinois, U.S.A.

[2] Porta Temp. Model 66590. Precision Scientific Co., Chicago Illinois, U.S.A.

Pig Experiment, Part 2: Finishing Phase

(a) Animals, Diets and Carcass Evaluation

At the end of an 11-week starting phase in Replicate 1, the pigs equalized as to sex, starting diets and the two major breeding groups (Pietrain-sired versus crossbred-sired pigs) were randomly allotted to two finishing diets, SBM- and PNM-supplemented diets respectively (Table 3). These diets were mixed from time to time as needed. Pigs in Replicate 1 averaged 20.8 (range: 7.1 - 41.1) kg at allotment. At the end of a 9-week starting phase in Replicate 2, the animals, which averaged 25.0 (range: 15.0 - 38.5) kg, were equalized as to starting diets and sex and randomly allotted to the two finishing diets.

The housing, pen arrangements and the routine of "feeding to appetite" thrice daily were as in the starting phase. Throughout the experiment, both in the starting and finishing phases, the individual pigs as well as their feed consumptions (FC) were weighed weekly and each pig marketed on the weigh day that it attained 87 kg liveweight. The slowest gaining pigs (4 in Replicate 1 and 3 in Replicate 2) were marketed at whatever weights they were at the time that the last of the other

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[1] Bendix International Operations, 605 Third Avenue, New York, N. Y. 10016, U.S.A.

TABLE 3: FORMULATION AND COMPOSITION OF FINISHING DIETS

Diet number Protein source[+]		11 SBM	12 PNM
Ingredients %			
Wheat (ground)		19.0	19.0
Barley (ground)		71.2	71.2
Soybean meal (48.5%)		7.0	--
Peanut meal (50.0%)		--	7.0
Iodized salt		0.5	0.5
Ground limestone		1.0	1.0
Calcium phosphate		0.3	0.3
Mineral-vitamin premix*		1.0	1.0
		100.0	100.0
Composition			
Digestible energy (calculated)	kcal/kg	3198	3207
Crude protein (average analysis)**	%	14.9	14.7
Lysine (analysis)	%	0.66	0.54
Calcium (calculated)	%	0.50	0.50
Phosphorus (calculated)	%	0.45	0.44

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* The premix provided the following trace minerals: 59 ppm zinc, 8 ppm manganese, 31 ppm iron, 2.7 ppm copper, and 0.3 ppm cobalt. It also provided per 100 kg diet: 293,000 I.U. Vitamin A, 37,000 I.U. Vitamin D, 370 I.U. Vitamin E, 0.30 g riboflavin, 0.60 g calcium pantothenate, 1.30 g niacin, 1.45 g choline chloride, 44 mg folic acid, and 0.3 µg Vitamin B_{12}. In addition, 50 g chlorotetracycline antibiotic was provided per 100 kg diet.

** Average of eight mixes analyzed in duplicate in each case.

[+] Hereafter, Diet 11 is referred to as SBM-supplemented finishing diet, and Diet 12 as PNM-supplemented finishing diet.

pigs reached 87 kg and the experiment terminated. All carcasses were graded by Canadian standards by the Hog Carcass Valuation system (CDA, 1969).

Rat Experiment

Eighty weanling Sprague - Dawley rats, The University of Alberta strain, were allotted to ten dietary treatments (Table 1) in two replicates of 40 rats each, equalized as to sex, in June and December 1972, respectively. Diets 1 - 8 were the same as for pigs, except that they were ground more finely and hand-mixed, whereas those for pigs were produced in a commercial-type feed mill at the University Edmonton Research Station. Diets 9 and 10 were additional to those used for pigs. In Replicate 1, the rats averaged 54.4 g at allotment and the total weight of rats on each treatment (average 217.7 g) were within 2 g of each other initially. The corresponding figures for Replicate 2 rats were 43.7 g, 174.8 g and 5 g.

The rats were kept in individual metal cages at a room temperature of approximately 23 C and relative humidity of 50 percent. Food and water were supplied ad libitum. Cages were cleaned weekly and liveweights (LW) and FC measured weekly also. To obtain accurate estimates of individual FC, spilled food was dried, weighed and accounted for.

The experimental period was 6 weeks at the end of which time the animals were killed with chloroform and the digestive tracts exposed mid-ventrally and freed of ingesta. The weights of individual ingesta-free carcasses were recorded immediately as empty body weights (EBW). Each carcass was then placed on ashless filter paper of known weight and oven-dried for 72 h at 60 C. Individual rat weights after equilibration with the atmosphere gave the air-dry EBW.

At the start of the experiment, eight representative rats (four males, four females) were killed as stated above and analyzed to obtain data to estimate the EBW, the crude protein (CP), crude fat (CF), dry matter (DM) and ash percentages of the rats at the beginning of the experiment. In Replicate 2 only, the rats were ground up at the end of the experiment and analyzed for CP, CF, DM, and ash; and the amount of energy stored as CP, CF, and in the entire carcass were estimated using the following equations:

Gross energy in carcass protein

$$(GE_P) = C_P \times W \times 5.65 \text{ kcal}$$

where C_P = % CP in individual carcass
W = DM weight of rat (g)
5.65 = kcal/g protein (NAS - NRC, 1966).

Gross energy in carcass fat

$$(GE_F) = C_F \times W \times 9.40 \text{ kcal}$$

where C_F = % CF in individual carcass
W = DM weight of rat (g)
9.40 = kcal/g fat (NAS - NRC, 1966).

Gross energy in rat carcass

$$(GE_R) = GE_P + GE_F$$

where GE_P and GE_F are as defined above.

In Replicate 2, immediately after killing the rats, the thyroid glands were removed and weighed, from which data, the mg thyroid per 100 g final LW were calculated. These thyroid glands were subsequently stained with Haematoxylin and eosin (Clayden, 1971) after 48 h fixation in 10% formaldehyde. For histological study, 6-micron sections were cut at four different levels.

Statistical Analysis

Except as otherwise specified, analyses of variance (ANOVA) and, where applicable, Duncan's multiple range test (Steel and Torrie, 1960), were used to detect significant differences. The sources of variation and levels for traits measured in the two species were as in Table 4. All sources of variation except replication and animals were considered as fixed. Because different numbers of breeds were used in each of the two replicates, the pig experiment was analysed separately for each replicate.

In Replicate 1, one pig sired by a crossbred boar died in the fourth week of the experiment. In order to analyze the metabolic data (measured only in Replicate 1), the resulting missing values were replaced with the average values of the remaining three pigs fed the same diet. Missing values for growth and feed consumption were replaced with the values for the corresponding two pigs from Replicate 2, as these traits were measured in both replicates.

In the finishing phase of Replicate 2, an attempt to distribute littermates equally to the two finishing diets resulted in the allotment of the two males from starting Diet 2 (PNM 50, SBM 50) to the SBM-supplemented finishing diet, and the allotment of the two males from starting Diet 7 (PNM 25, RSM 75) to the PNM-supplemented finishing diet, thus leaving two empty cells. Both in LW and other traits, the resulting missing figures and those due to the one death in Replicate 1 were estimated using the covariance procedure for missing data (Katti, 1965). To adjust for the variation in LW at the commencement of the finishing phase, the covariance analyses (Steel and Torrie, 1960) with weight as covariate was then performed on the growth data. The carcass data were subjected to ANOVA, after substituting the missing data.

Because of observed variation in the pattern of plasma urea concentrations at the various intervals within and between replicates, it was

TABLE 4: SOURCES OF VARIATION AND NUMBER OF LEVELS FOR EACH OF THE TRAITS

Species	Phase and/or replication	Traits	Diet	Sex	Breed	Age (weeks)	No. of bleedings	Replication	Observations per treatment by sex combination
Pigs	Starting phase.	Growth and feed consumption (Tables 5, and 6.)	8	2	2 1 [*]	9 8 [*]	NA	1	1 2 [*]
	Replicates 1 and 2, respectively.	Average daily feed Average daily gain kg feed/kg gain							
		Metabolism studies (Table 7)	8	2	2	NA	NA	1	1
		Energy and nitrogen (N) digestibility and retention							
		Blood plasma measurements							
		Plasma urea (Table 8)	8	2	2 1 [*]	NA	7	2	1 or 2[+] 2 [*]
		Plasma amino acids (Tables 11 and 12).	8	P	P	NA	5	2	1
	Finishing phase.	Days to market, feed consumption and gain (Table 13)							
	Replicates 1 and 2, respectively.	Days to market Average daily feed Average daily gain kg feed/kg gain	2	2	2 1 [*]	NA	NA	1	1 2 [*]
		Carcass analyses (Table 14)							
		Market weight (kg) Carcass weight (kg) Dressing (%) Total backfat (cm) Length of side (cm) Lean in ham face (%) Ham wt./carcass wt. (%) Grade index ROP index	2	2	2 1 [*]	NA	NA	1	1 2 [*]
Rats		Growth and food (Table 15)							
	Replicates 1 and 2 combined.	Food, gain and g food/g gain	10	2	1	6	NA	2	2
		Carcass Analyses (Table 16)							
		Carcass ash % Carcass crude protein % Carcass crude fat % kcal in carcass protein kcal in carcass fat kcal in entire carcass mg thyroid/100 g liveweight	10	2	1	NA	NA	1	2

[*] The upper-values are for replicate 1, the lower for replicate 2.

[NA] Not applicable.

[P] Blood plasma samples were pooled; 5 ml from each donor, three donors in replicate 1, four donors in replicate 2.

[+] Three largest pigs were bled; two males and one female or one male and two females.

necessary to use total urea output, rather than relative peak values at any given time. Total urea output for each pig was calculated from the relationship:

$$U = PV \times W \times [u] \text{ ....} (1)$$

where U = Total urea output (g)

PV = Blood plasma per kg body weight (ml)

W = Body weight of individual pig (kg)

[u] = Average mg urea/100 ml plasma determined at 0, 1, 2, 4, 5 and 7 h postprandial.

10^{-5} = Factor for converting mg% urea to g urea/ml plasma.

An equation for estimating PV for the various pig weights was obtained by fitting a semi-log transformation to the data of Bush et al. (1955) in the form of:

$$PV = A + B \ \ell n \ (W)$$

whence A = 86.26

B = -11.07

ℓn = Natural log, and PV and W are as defined above.

Fitting this equation $PV = 86.26 - 11.07 \ \ell n \ (W)$ to the data of Bush et al. (1955) gave an R^2 of 0.98. As the pig weights (10 - 50 kg) encountered in the present study were within the range (10 - 110 kg) studied by Bush et al. (1955), it was felt that the PV values in the present study could be accurately predicted using this equation.

The N intake of each pig was calculated as:

$$\text{N intake} = \text{Feed intake on morning of blood sampling (g)} \times \text{dietary crude protein \%} \times 0.0016 \text{..} (2)$$

where 0.0016 = Average content of N per g feed.

From equations (1) and (2) above, the ratio U/N intake i.e. the urea output per unit intake of N for the various treatments were calculated with and without correction for the values at 0 h.

For the plasma amino acids, peak values and peaking times were considered more critical than total amino acid outputs over the observation span because of the dependence of effective protein synthesis on the simultaneous supply of all needed amino acids at the sites of synthesis. Quadratic equations of the form:

$$Y = A + b_1 x + b_2 x^2$$

where Y = Amino acid of interest

x = Blood sampling time in h

were estimated for each replicate by treatment combination. From the calculated equations, the peaking times and maximum values were estimated for each of the replicate by treatment combinations. Some equations resulted in peaking times and maximum peaks beyond the range actually observed. In those cases, the predicted values were replaced with observed values for ANOVA.

For the purpose of graphing the relative plasma lysine peaks at various postprandial intervals (Figure 1), average values were used. Based on similarity of pattern, the values of Diets 1 (SBM 100), 2 (PNM 50, SBM 50) and 4 (PNM + lysine); those of Diets 6 (PNM 50, RSM 50), 7 (PNM 25, RSM 25) and 8 (RSM 100) and those of Diets 3 (PNM 100) and 5 (PNM 75, RSM 25) were averaged together. These averages were used to generate a theoretical curve of the form:

$$Y = A + Bx + Cx^2 + Dx^3$$

where Y = Postprandial lysine concentration (μm %)

x = Blood sampling time in h.

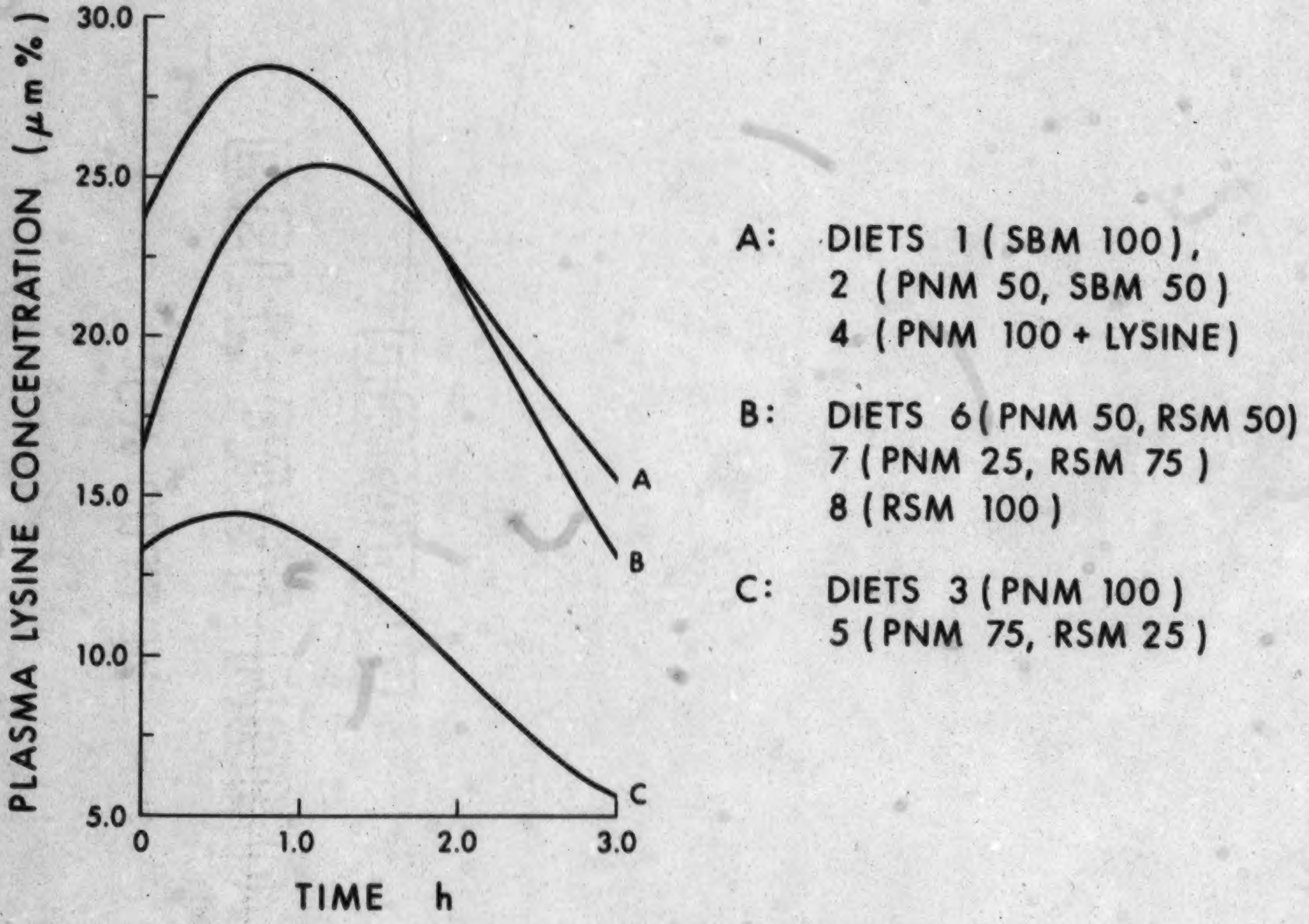

Figure 1: Postprandial lysine concentrations.

The results are presented and discussed in the same sequence as traits are listed (Table 4). Where data from replicate trials could not be pooled together statistically as occurred in some traits in the pig experiment, the results are presented chronologically by replicate, followed by an overall interpretation of the two replicates. In the rat experiment, the growth and food consumption data are pooled; carcass analyses are based on Replicate 2 only.

Table 5: Symbols used in significance tests

Symbols	Meaning
*	The two means compared are significantly different at $P \leq 0.05$.
**	The two means compared are significantly different at $P \leq 0.01$.
a, b, c, d, e,	Means of the same series (column or row) bearing the same letter or no letter are not significantly different at $P \leq 0.01$.
A, B, C, D, E,	Means of the same series (column or row) bearing the same letter or no letter are not significantly different at $P \leq 0.05$.

RESULTS AND DISCUSSION

PIG EXPERIMENT, STARTING PHASE, REPLICATES 1 AND 2.

Average daily feed (ADF), average daily gain (ADG) and efficiency of feed conversion (EFC) (Tables 5 and 6).

During the first 10 weeks of the experiment, in both Replicates 1 and 2, the best ADF, ADG and EFC were shown by pigs fed Diet 1 (SBM 100). However, in Replicate 1, the ADF of pigs fed the control Diet 1 was significantly different ($P \leq 0.01$) from those of pigs consuming all diets except Diets 2 (PNM 50, SBM 50), 4 (PNM 100 + lysine) and 7 (PNM 25, RSM 75). In Replicate 2, it was only statistically superior ($P \leq 0.01$) to those of pigs fed Diets 3 (PNM 100) and 5 (PNM 75, RSM 25). In ADG, the only diets that produced growth statistically equal ($P \leq 0.01$) to those of pigs on the control diet were Diets 2 (PNM 50, SBM 50) in Replicates 1 and 2 and Diet 8 (RSM 100) in Replicate 2. In Replicate 1, the only pigs which had an EFC statistically equal ($P \leq 0.01$) to those of the pigs on the control diet were those fed Diet 2 (PNM 50, RSM 50). In Replicate 2, the EFC of the control pigs was only statistically different ($P \leq 0.01$) from those of pigs on Diets 3 (PNM 100), 5 (PNM 75, RSM 25) and 6 (PNM 50, RSM 50).

Although the statistical results are variable, a summary of these performance data suggests that the substitution of PNM for 50% of the SBM reduced ADF and lowered ADG but had little effect on EFC. Use of 100% PNM had a very depressive effect on performance. The addition of lysine to the PNM-supplemented diet resulted in performance similar to that obtained from a 50-50 mixture of SBM-PNM.

Replacement of 25% of the PNM with RSM did not alter performance.

TABLE 5: AVERAGE DAILY FEED (ADF), AVERAGE DAILY GAIN (ADG) AND EFFICIENCY OF FEED CONVERSION (EFC) FOR PIGS, REPLICATE 1

Diet no.	Supplemental protein source(s)%	ADF kg	ADG kg	EFC kg feed /kg gain
1	SBM 100	0.86^{a}	0.45^{a}	1.93^{a}
2	PNM 50, SBM 50	0.66^{ab}	0.32^{ab}	2.06^{b}
3	PNM 100	0.36^{b}	0.15^{c}	2.53^{de}
4	PNM 100 + lysine	0.60^{ab}	0.28^{bc}	2.24^{c}
5	PNM 75, RSM 25	0.32^{b}	0.13^{c}	2.61^{e}
6	PNM 50, RSM 50	0.40^{b}	0.17^{bc}	2.41^{d}
7	PNM 25, RSM 75	0.59^{ab}	0.28^{bc}	2.12^{bc}
8	RSM 100	0.50^{b}	0.24^{bc}	2.20^{c}
Sex				
	Barrows	0.52	0.24	2.36*
	Gilts	0.56	0.27	2.17
Breed				
	Pietrain-sired pigs	0.49	0.23	2.22
	Crossbred-sired pigs	0.59	0.27	2.30
Grand mean		0.54	0.25	2.26
Standard error of mean		0.07	0.03	0.03

TABLE 6: AVERAGE DAILY FEED (ADF), AVERAGE DAILY GAIN (ADG), AND EFFICIENCY OF FEED CONVERSION (EFC) FOR PIGS, REPLICATE 2

Diet no.	Supplemental protein source(s)%	ADF kg	ADG kg	EFC kg feed /kg gain
1	SBM 100	1.15^{a}	0.57^{a}	2.03^{a}
2	PNM 50, SBM 50	0.97^{ab}	0.44^{ab}	2.21^{ab}
3	PNM 100	0.55^{c}	0.20^{d}	2.67^{c}
4	PNM 100 + lysine	0.90^{abc}	0.40^{bc}	2.27^{ab}
5	PNM 75, RSM 25	0.65^{bc}	0.28^{cd}	2.37^{b}
6	PNM 50, RSM 50	0.80^{abc}	0.35^{bcd}	2.29^{b}
7	PNM 25, RSM 75	0.90^{abc}	0.40^{bc}	2.27^{ab}
8	RSM 100	0.96^{ab}	0.43^{abc}	2.23^{ab}
Sex				
	Barrows	0.84	0.38	2.25
	Gilts	0.88	0.38	2.34*
Grand mean		0.86	0.38	2.29
Standard error of mean		0.09	0.04	0.05

Replacement of 50% of the PNM with RSM resulted in improved ADG. Replacement of 75% of the PNM with RSM resulted in ADF, ADG and EFC almost identical to those obtained when 100% PNM was supplemented with lysine and much superior to straight PNM as a supplement. Use of 100% RSM resulted in no further improvement over that with a mixture of 25% PNM-75% RSM.

The amino acid assays (Table 2) indicate that Diet 1 (SBM 100) had higher absolute amounts of most amino acids, although compared with NAS-NRC requirements (1968), only the differences in lysine, methionine-cystine and possibly threonine would be crucial. These were the only amino acids in which dietary levels were below the recommended levels for pigs which at allotment to starting diets averaged 5 - 10 kg liveweight. The methionine-cystine contents of Diets 3 (PNM 100) and 5 (PNM 75, RSM 25) were almost identical with that of the control diet. It would appear that although methionine-cystine levels may be suboptimal in these diets, lysine is the most limiting factor. This view is supported by the close similarity in performance of pigs fed Diet 3 (PNM 100) and Diet 5 (PNM 75, RSM 25). These two diets which yielded non-statistically different (P [illegible] 0.01) performances (ADF, ADG and EFC) each had 0.65% dietary lysine.

Although with the exception of pigs fed the control diet there was statistically no significant difference in ADF between pigs consuming Diets 3 (PNM 100) and 5 (PNM 75, RSM 25) respectively compared with the rest, the observed variations in ADF among diets are important, in view of the possible effects of ADF on ADG. Kumta and Harper (1961) working with rats fed _ad libitum_ associated depressed food intake with amino acid imbalance. In the present study, supplementation of Diet 3

(PNM 100) with lysine i.e. Diet 4 (PNM 100 + lysine) improved ADF, ADG and EFC; although only the last of these was significantly different ($P \leq 0.01$). It would appear, therefore, that pigs, like rats, are biologically sensitive to amino acid imbalance, especially imbalance associated with lysine insufficiency (Table 2), and will reduce feed consumption with associated effects on ADG. At a time of high cost of protein supplements, this has serious implications for the pig producer.

Replacement of 50% of the PNM with RSM altered the dietary levels of some essential amino acids as follows: arginine - 0.27%, leucine - 0.02%, lysine + 0.09%, methionine-cystine - 0.08%, phenylalanine - 0.07% and threonine + 0.06%. These alterations may have been associated with the insignificant improvements in ADF, ADG and EFC of Diet 6 (PNM 50, RSM 50) compared with Diets 3 (PNM 100) and 5 (PNM 75, RSM 25). From the foregoing discussion, it is likely that of these changes in dietary amino acid levels, the difference in lysine accounts for much of the increased response. Full replacement of PNM with RSM did not give any further increased advantage in ADF, ADG and EFC, compared with 75% replacement. This diet with 100% RSM did not result in numerical performance equal to Diet 1 (SBM 100), even though lysine levels were similar. This suggests that either availability of lysine in RSM is not equal to that in SBM or that some other essential amino acid is limiting in RSM.

Though gilts were slightly better than barrows in ADF, ADG and EFC, in Replicate 1, the only significant difference ($P \leq 0.05$) was in EFC. This trend in gain is different from that of Bowland (1971), who found that barrows ate more, gained more and required more feed per kg gain than gilts. In Replicate 2, there were no significant differences between barrows and gilts in ADF and ADG, although gilts tended to be better. The superiority in EFC ($P \leq 0.05$) recorded for gilts in Replicate 1 was reversed in Replicate 2. With young pigs as used in the present study it is not unusual for gilts to gain as rapidly as barrows and to be similar in EFC. For example, Standish and Bowland (1967) observed similar relationships between sexes to that observed in Replicate 1.

There were no significant breed effects on ADF, ADG and EFC. Pigs sired by crossbred boars averaged 0.59 kg ADF, 0.27 ADG, and 2.30 kg feed per kg gain, compared with 0.49 kg ADF, 0.25 kg ADG and 2.22 kg feed per kg gain for those sired by a Pietrain boar. Results of the ANOVA analyses indicated a highly significant diet X breed effect. However, the absence of consistent trends suggested that the small number of animals per diet by breed combination might in most cases be responsible for the apparent significance. This makes it difficult to assess what diet X breed interactions might exist.

Although the effect of replication could not be statistically assessed, as the same breeds were not featured in the two replications, Replicate 2 pigs generally performed better than those of Replicate 1, especially in ADF and ADG. ADF, ADG and average EFC for Replicate 2 pigs were 0.86 kg, 0.38 kg and 2.29 kg feed per kg gain compared with 0.54 kg, 0.25 kg and 2.25 kg feed per kg gain for Replicate 1 pigs. However, the trends and pattern of significant differences in the two replicates were similar.

An overview of the results of the two replicates would support the thesis that:

1. For starting pigs, prepress solvent-extracted Span RSM is

superior to solvent-extracted PNM.

2. Prepress solvent-extracted Span RSM will beneficially supplement PNM, probably due to the superiority of Span RSM in lysine.

Energy and nitrogen (N) digestibilities and retention (Table 7).

The control SBM-supplemented Diet 1 had a digestible energy (DE) coefficient of 89.7%. This was the highest DE but significantly different ($P \leq 0.05$) only from the 81.6% DE in Diet 6 (PNM 50, RSM 50) and 81.3% DE in Diet 7 (PNM 25, RSM 75). The metabolizable energy (ME), the N-corrected metabolizable energy (ME_n) percentages and the kcal ME/kg feed followed similar patterns to percentage DE. Together, they suggest that both PNM and RSM are lower in DE, ME, and ME_n than SBM. Compared with SBM, a relatively low DE or ME value for RSM but not for PNM is consistent with the literature evidence (NAS - NRC, 1968; Saben, et al., 1971). But as pointed out by March et al. (1972), many factors among which the age and the breed of the animal used for the bioassay can affect the ME, DE or ME_n value. Regardless of the form in which the energy digestibility is expressed, lysine supplementation of Diet 3 (PNM 100) considerably enhanced energy utilization.

Although Pietrain-sired pigs used energy slightly more efficiently than crossbred-sired pigs, and gilts slightly better than barrows, neither sex nor breed had any significant influence on energy utilization.

DN percentage was lowest ($P \leq 0.05$) at 85.7% in Diet 5 (PNM 75, RSM 25), although only significantly lower than the DN of the control (93.0%) and of Diet 2 (PNM 50, SBM 50) (91.5%). Lysine supplementation of the PNM-supplemented diet raised the dietary level from 0.65 to 0.76% which

TABLE 7: ENERGY AND NITROGEN (N) DIGESTIBILITIES AND RETENTION[1]

Diet no.	Supplemental protein source(s) %	DE		ME		ME_n	DN		NR		NR/DN
		%	kcal/kg	%	kcal/kg	%	%	g/kg	%	g/kg	%
1	SBM 100	89.7^{A}	3541	86.8^{A}	3426^{AB}	85.1^{A}	93.0^{A}	26.8^{AB}	59.0^{A}	17.0^{A}	63.5^{A}
2	PNM 50, SBM 50	89.0^{AB}	3586	85.9^{AB}	3459^{A}	83.9^{AB}	91.5^{AB}	27.2^{AB}	51.7^{ABC}	15.4^{AB}	51.2^{AB}
3	PNM 100	83.0^{AB}	3397	79.3^{BC}	3244^{AB}	76.4^{C}	86.3^{BC}	25.1^{C}	42.4^{BC}	12.1^{B}	49.0^{B}
4	PNM 100 + lysine	88.7^{AB}	3636	86.1^{AB}	3529^{A}	84.3^{AB}	91.2^{ABC}	27.3^{A}	54.5^{AB}	16.3^{A}	59.8^{AB}
5	PNM 75, RSM 25	82.0^{AB}	3354	79.2^{BC}	3241^{AB}	77.4^{BC}	85.7^{C}	25.0^{C}	47.4^{ABC}	13.8^{AB}	54.6^{AB}
6	PNM 50, RSM 50	81.6^{B}	3270	78.3^{C}	3138^{B}	76.4^{C}	86.1^{BC}	25.7^{BC}	49.3^{ABC}	14.7^{AB}	57.4^{AB}
7	PNM 25, RSM 75	81.3^{B}	3404	77.2^{C}	3153^{B}	74.9^{C}	87.7^{ABC}	25.9^{ABC}	40.9^{C}	12.1^{B}	46.1^{B}
8	RSM 100	85.0^{AB}	3449	82.2^{ABC}	3334^{AB}	80.6^{ABC}	88.6^{ABC}	26.2^{ABC}	57.1^{A}	16.9^{A}	64.5^{A}
Sex											
	Barrows	84.2	3410	80.9	3277	78.9	87.8	25.8	47.0	13.8	52.0
	Gilts	85.9	3499	82.8	3354	81.0	89.8	26.5	53.6*	15.7*	59.6*
Breed											
	Pietrain-sired pigs	85.9	3481	82.8	3356	81.0	89.0	26.1	51.0	15.0	55.7
	Crossbred-sired pigs	84.1	3428	80.9	3275	78.8	88.6	26.1	49.6	14.6	55.8
Grand mean		85.0	3454	81.9	3315	79.9	88.8	26.1	50.3	14.8	55.8
Standard error of mean		2.2	110	2.0	83	2.0	1.6	0.4	3.5	1.0	3.9

[1] Abbreviations: DE = Digestible energy; ME = Metabolizable energy; ME_n = Nitrogen-corrected metabolizable energy; DN = Digestible nitrogen; NR = Nitrogen retained.

is intermediate between the 0.74% lysine in Diet 6 (PNM 50, RSM 50) and the 0.78% in Diet 2 (PNM 50, SBM 50), and is considerably less than the 0.83% lysine in Diet 7 (PNM 25, RSM 75). However, N digestibility and retention (whether absolute values or coefficients) were on the whole as good ($P \leq 0.05$) for these diets as for Diet 2 (PNM 50, SBM 50) and in some cases slightly better.

Raising the dietary lysine level in PNM-supplemented diets to a level similar to that in Diet 4 (PNM 100 + lysine) by the use of RSM would be expected to produce improvements in N digestibility similar to those in Diet 4 (PNM 100 + lysine), but, it did not. Low energy values of RSM cannot be associated with this result for a comparison of the DE and ME of Diet 8 (RSM 100) and 3 (PNM 100) does not suggest that supplementation of wheat-barley diet with Span RSM yields lower energy values than when the same basal diet is supplemented with PNM. A possible conclusion from this is that the lysine in Span RSM is neither as available to starting pigs as the lysine in SBM nor as the synthetic supplemental lysine.

Breed had no significant effect on N digestibility and retention. Gilts were superior ($P \leq 0.05$) to barrows in their N retention (NR%, NR/DN% and g N retained/kg feed ingested). Gilts have long been known to be leaner than barrows (Woodman _et_ _al_., 1937; McMeekan, 1940c). Many more recent studies, for example, Pierce and Bowland (1972) have confirmed this relationship. In the present study, the observed superior N retention of gilts relative to barrows demonstrates this fact.

Plasma urea measurements (Table 8).

A comparison of the dietary amino acid composition (Table 2) with

TABLE 8: PLASMA UREA CONCENTRATION OF PIGS AND PERCENTAGE INCREASE IN PLASMA UREA OUTPUT PER g N INGESTED*

Diet no.	Supplemental protein source(s)%	mg urea /100 ml plasma (0-7h)	Increase in urea output per N ingested* (%) (0-7h)	(0-3h)	(4-7h)
1	SBM 100	41.8^{a}	0.8^{B}	1.1^{B}	0.5
2	PNM 50, SBM 50	39.6^{ab}	0.9^{B}	0.8^{B}	0.9
3	PNM 100	38.7^{abc}	2.9^{A}	2.3^{A}	3.5
4	PNM 100 + lysine	38.1^{abc}	1.2^{B}	0.9^{B}	1.6
5	PNM 75, RSM 25	34.1^{abcd}	1.6^{AB}	1.6^{AB}	1.7
6	PNM 50, RSM 50	30.0^{bcd}	1.3^{B}	1.1^{B}	1.4
7	PNM 25, RSM 75	30.6^{cd}	1.0^{B}	1.0^{B}	0.9
8	RSM 100	27.9^{d}	1.2^{B}	1.1^{B}	1.4
Grand mean		35.1	1.4	1.2	1.5
Standard error of mean		4.4	0.4	0.3	0.6

* Nitrogen (g) ingested in feed on morning of blood sampling.

the amino acid requirements of the pig (NAS - NRC, 1968) suggested that Diet 3 (PNM 100) and Diet 5 (PNM 75, RSM 25), containing only 0.65% lysine compared with 0.92% lysine in the control Diet 1 (SBM 100) would be the most limiting in growth. Actual growth (Table 5) supported this view; but regular ANOVA of the urea data showed that the pigs fed the control diet had the highest average concentration of plasma urea. This was contrary to initial expectation and the findings of other investigators (Brown and Cline, 1972; Eggum, 1970; Kornegay, 1972), who reported an inverse relationship of plasma urea concentration to dietary protein quality, but results may be related to the high lysine level in Diet 1 (SBM 100).

Eggum (1970) working with one pig cathetherized in the portal vein and fed a barley and casein diet noted that 3 - 4 h may elapse before blood urea content is constant. He therefore suggested that 4 - 5 h after withdrawal of food should be a suitable time-period for a standardized test. In the present study, in which blood samples were collected for a period of 7 h compared with 5 h total collection period used by Eggum (1970), plasma urea levels continued to rise, in some cases exhibiting a marked variation both in the absolute concentrations and pattern of rise among diets. The concentrations averaging 25 - 45 mg per 100 ml plasma were in agreement with those of Veum _et al_. (1970) and McCance, (1960). In the present study, although plasma urea concentrations on the whole rose for the first 2 h and peaked initially at 3 h postprandial, subsequent rise and fall in urea concentrations varied, with the plasma urea concentration in some pigs still rising, others falling at 7 h. The variation among diets in the pattern of urea output was not surprising, since the many factors which affect the rates of stomach emptying (Porter and Rolls, 1971) could conceivably effect different rates of urea production in animals consuming different diets. In the absence of a common peaking time for all the diets, performing the ANOVA on the basis of the percentage increase in urea output for the periods 0 - 3 h, 4 - 7 h and 0 - 7 h respectively per g N ingested on the morning of blood sampling seemed logical.

The overall (0 - 7 h) percentage increase in urea output for N ingested on the morning of blood sampling and similar calculations based on measurements taken 4 - 7 h gave similar and the most meaningful ranking of the diets. However, in the latter case i.e. when the comparisons were based on blood samples taken from 4 - 7 h postprandial, there were statistically no significant differences between diets in the percentage increase in urea output per N ingested. On the basis of the 0 - 7 h percentage increase in urea output per N ingested, the ranking of the diets in descending order was 1, 2, 7, 4, 8, 6, 5 and 3, compared with 1, 2, 7, 8, 6, 4, 5, and 3 when the statistical analyses were based on measurements taken during the period 4 - 7 h and 2, 4, 7, 1, 6, 8, 5 and 3 for measurements taken from 0 - 3 h postprandial. These rankings compare with 1 (SBM 100), 2 (PNM 50, SBM 50), 4 (PNM 100 + lysine), 7 (PNM 25, RSM 75), 8 (RSM 100), 6 (PNM 50, RSM 50), 5 (PNM 75, RSM 25) and 3 (PNM 100) when the ranking was based on liveweight gain. The least meaningful ranking 1, 2, 3, 4, 5, 7, 6 and 8 was obtained when statistical analyses were based on average urea concentrations for the period 0 - 7 h.

On the basis of the cumulative (0 - 7 h) increase in urea output (U/N%), the control (SBM 100) diet was the best ($P \leq 0.05$) but not significantly different from Diets 2 (PNM 50, SBM 50), 7 (PNM 25, RSM 75), 4 (PNM 100 + lysine) and 8 (RSM 100). Diet 3 (PNM 100) was the worst ($P \leq 0.05$), but not significantly different from 5 (PNM 75, RSM 25). There were no significant differences between Diets 2 (PNM 50, SBM 50), 4 (PNM 100 + lysine), 5 (PNM 75, RSM 25), 6 (PNM 50, RSM 50), 7 (PNM 25, RSM 75) and 8 (RSM 100). This procedure ranked the diets similar to the ranking arrived at from liveweight gains (Tables 5 & 6). Statistically, both the urea and liveweight techniques could identify the best and the worst diets; but there was a tendency in both, especially the urea procedure, to group the intermediate diets together, when conclusions were based on statistical differences rather than mere sequence or trend. This study suggests that measuring the relative increase in urea output per unit N ingested can enhance the precision of the plasma urea criterion in assessing dietary protein quality.

Several investigators have observed that the pig can utilize a small amount (1 - 2%) of urea (Grimson _et al_., 1970; Kornegay _et al_., 1970), especially in dietary situations in which the essential amino acid requirements were met and insufficiency of total N was limiting growth (Kornegay, 1972). Some investigators (Hintz _et al_., 1969) have attributed the slight utilization of urea in pigs to ureolytic activities occurring mainly in the caeca and colon of pigs. They argued that the restriction of ureolytic activity to the area beyond the site of maximal amino acid absorption would limit the availability to the host of the amino acids synthesized by the microbes, unless coprophagy occurs. However, Grimson _et al_. (1970) have reported the incorporation of ^{15}N-of dietary ^{15}N-urea into the trichloroacetic acid - insoluble fraction of liver, blood plasma, intestinal mucosa, skeletal muscle and blood cells of 10 kg weanling pigs.

Physiologically, it is known that about 40 - 50% of urea is reabsorbed by the kidney tubules (Guyton, 1969); and that apart from dietary protein level and quality, factors such as renal function and water consumption affect the rate of urea excretion and absorption. Moreover, the presence in the diet of the tetracycline group of antibiotics can produce an elevated blood urea concentration (Anon., 1969).

Eggum (1970) emphasized the need to standardize the conditions other than protein source when using blood urea as a criterion for assessing protein quality. In the present study, an attempt to give the animals a regulated amount of feed on the morning of blood sampling was impracticable because some pigs would not consume their full quota.

Lack of a consistent inverse relationship between plasma urea concentration and protein quality when calculations were made without considering feed intake could therefore be due to the fact that neither feed nor water were standardized in this study. Furthermore, the diets used were such that their respective impacts on plasma urea outputs could not be fully measured within the 7 h observation span. Another factor which might conceivably modify the plasma urea concentrations in pigs is urea recycling and utilization. These factors depend on many variables, among which are the kidney status and the degree of essential amino acid deficiency.

Urea recycling, differential utilization of urea depending on dietary amino acid and total N supply, the exposure of the animals for long periods (8 - 9 weeks) to the experimental diets before blood sampling and the varied adaptation of the animals to the different diets could all combine to make it difficult to directly correlate postprandial plasma urea concentration with protein quality, especially in diets such as used in this study. The fact that the total urea output expressed as a percentage of dietary N intake, after correction for the initial concentrations, gave the most meaningful trend, supports the view that standardized conditions are essential.

Amino acid composition of diets (Table 2).

Jones (1969) made the point that protein concentrates vary in quality because they differ in their ability to supply the lysine requirements of the pig. He therefore suggested that an analysis for lysine in protein concentrates would be a better guide to their biological value (BV) than an analysis for crude protein. If in the present study, lysine is regarded as the first limiting amino acid, then it would be apparent from Table 2 that Diet 8 (RSM 100) with a lysine level of 0.88% and Diet 7 (PNM 25, RSM 75) with a lysine level of 0.83% should each rival closely the control diet in nutritive value. This was, in fact, not the case. Pigs fed Diet 8 (RSM 100) which was second in lysine content to the control diet ranked fifth in performance. Pigs fed Diet 7 (PNM 25, RSM 75), third to the control diet in lysine content, were fourth in performance. Diet 2 (PNM 50, SBM 50), fourth to the control diet in lysine content, ranked second to the control diet in promoting performance. While not precluding the possibility of lysine limitation, the above observations indicate that amino acid analysis, much as it represents advancement over the previous reliance on dietary crude protein analysis, is not necessarily a measure of what use the animal can make of the amino acids assayed in the diets. This stresses the need for a rapid, reliable procedure for determining the availability of individual essential amino acids.

From the observed lack of direct correlation between the dietary lysine levels and the biological performances in the present study, it is apparent that essential amino acids other than lysine might be first limiting. Therefore, a thorough probe into the sequence of limiting amino acids in the various diets is necessary. The concept of chemical score (Block and Mitchell, 1946), whereby the amino acid of a test protein is related to that of a standard protein, usually whole egg, or to the amino acid requirements of the species concerned, theoretically measures how good the test protein is compared with the standard protein, or how well it can meet the amino acid requirements of the species concerned. It also indicates the sequence of limiting amino acids.

Using the amino acid composition of whole egg as the standard in determining the chemical score (Table 9), it appears that in all eight diets, isoleucine is the first limiting amino acid for the pigs, followed by lysine in some diets and by methionine-cystine in others. The isoleucine requirements for 5 - 10 kg pigs is reported to be 0.76% (NAS-NRC, 1968). Dietary levels vary from 0.63% in Diet 5 (PNM 75, RSM 25) to 0.78% in the control Diet 1. It is therefore, probable that the chemical score with egg protein as a standard overestimates the isoleucine requirement of 5 - 10 kg pigs, as it indicates that isoleucine is the first limiting amino acid in the control diet which, in fact, contains more than the NAS - NRC (1968) requirement. The reliability of the chemical score using egg protein as the standard is therefore questionable.

On the basis of average liveweight gain per replicate and in a descending order of superiority, the diets ranked as follows: Diet 1 (SBM 100) 31.6 kg; 2 (PNM 50, SBM 50) 23.8 kg; 4 (PNM 100 + lysine) 21.1 kg; 7 (PNM 25, RSM 75) 20.9 kg; 8 (RSM 100) 20.8 kg; 6 (PNM 50, RSM 50) 16.1 kg; 5 (PNM 75, RSM 25) 12.7 kg and 3 (RSM 100) 11.0 kg. Determination of the chemical score (Table 10) on the basis of NAS-NRC (1968) requirements indicates that lysine is the first limiting amino acid in all eight diets, followed by methionine-cystine in Diets 1 (SBM 100),

TABLE 9: CHEMICAL SCORE OF PIG DIETS BASED ON WHOLE EGG PROTEIN

		Starting Diets (%)*								Finishing Diets (%)*		Amino+ acid in whole egg %
Diet number		1	2	3	4	5	6	7	8	11	12	
Supplemental protein source(s) %	PNM	---	50	100	100	75	50	25	---			
	SBM	100	50	---	+	---	---	---	---	SBM	PNM	
	RSM	---	---	---	lysine	25	50	75	100			
Essential amino acids												
Arginine		90.5	107.9	124.3	120.6	111.9	101.1	91.2	80.2	87.6	105.5	6.4
Histidine		115.5	113.8	112.6	106.4	114.8	111.7	116.4	121.2	113.6	109.0	2.1
Isoleucine		49.3	44.6	42.8	41.1	42.9	42.9	43.6	45.1	46.2	43.1	8.0
Leucine		74.9	71.0	71.0	67.6	69.8	69.2	70.2	71.2	74.6	70.8	9.2
Lysine		64.6	55.8	47.1	54.6	49.7	53.1	58.9	63.6	61.6	51.0	7.2
Methionine-cystine		51.4	47.8	56.2	52.3	53.4	44.8	59.0	62.3	61.9	54.3	6.5
Phenylalanine-tyrosine		66.0	65.7	68.0	65.5	65.3	62.3	61.4	58.8	64.8	65.1	10.8
Threonine		69.9	63.2	59.5	56.0	61.4	65.1	69.1	74.0	69.5	62.4	4.9
Valine		61.5	57.7	58.9	55.5	58.8	58.6	60.2	61.9	65.1	62.1	7.3

* Amino acid of diet as percentage of corresponding amino acid in whole egg.

+ Source: Block and Mitchell (1946).

TABLE 10: CHEMICAL SCORE OF PIG DIETS BASED ON NRC REQUIREMENTS

		Starting Diets (%)*									Finishing Diets(%)*		
Diet number		1	2	3	4	5	6	7	8	NRC	11	12	NRC
Supplemental protein source(s) %	PNM	---	50	100	100	75	50	25	---				
	SBM	100	50	---	+	---	---	---	---	Requirement %	SBM	PNM	Requirement %
	RSM	---	---	---	lysine	25	50	75	100				
Essential amino acids													
Arginine		----	----	----	----	----	----	----	----	----	415.0	495.0	0.20
Histidine		177.8	170.4	170.4	159.2	163.0	170.4	177.8	181.5	0.27	194.4	188.9	0.18
Isoleucine		102.6	90.8	86.8	84.2	82.9	86.8	89.5	90.8	0.76	110.0	102.0	0.50
Leucine		151.1	140.0	140.0	134.4	130.0	137.8	140.0	140.0	0.90	170.0	160.0	0.60
Lysine		76.7	65.0	54.2	63.3	54.2	61.7	69.2	73.3	1.20	94.3	77.1	0.70
Methionine-cystine		82.5	75.0	81.2	82.5	78.8	71.2	93.8	96.2	0.80	118.0	104.0	0.50
Phenylalanine-tyrosine		----	----	----	----	----	----	----	----	----	208.0	206.0	0.50
Threonine		97.1	85.7	80.0	75.7	78.6	88.6	94.3	100.0	0.70	111.1	100.0	0.45
Valine		135.4	126.2	127.7	121.5	120.0	127.7	132.2	133.8	0.65	142.0	134.0	0.50

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*Amino acid of diet as percentage of the requirement (NAS - NRC, 1968) for each amino acid.

2 (PNM 50, SBM 50) and 6 (PNM 50, RSM 50), by threonine in Diets 3 (PNM 100), 4 (PNM 100 + lysine) and 5 (PNM 75, RSM 25) and by isoleucine in Diets 7 (PNM 25, RSM 75) and 8 (RSM 100). Only in Diets 7 (PNM 25, RSM 75) and 8 (RSM 100) do the two standards identify isoleucine as a common limiting amino acid. The isoleucine content of Diet 4 (PNM 100 + lysine), 0.64% is less than that of Diets 3 (PNM 100), 7 (PNM 25, RSM 75) and 8 (RSM 100) containing 0.66%, 0.68% and 0.69% isoleucine respectively. However, the pigs fed Diet 4 (PNM 100 + lysine) averaged 21.0 kg gain per replicate, while those fed Diets 3 (PNM 100), 7 (PNM 25, RSM 75) and 8 (RSM 100) averaged 11.0, 20.9 and 20.8 kg gain respectively. These trends suggest that the differences in metabolically useful dietary lysine may explain more of the observed differences in gain than the differences in dietary isoleucine. The net implication therefore appears to be that the chemical score calculated on the basis of NAS-NRC (1968) requirements for 5 - 10 kg pigs is a better indicator of the first limiting amino acid in these diets than the chemical score based on the amino acid composition of whole egg. This does not mean that if used in calculating the chemical score for other species, the amino acid requirements of the pig would be as reliable nor the amino acid composition of whole egg as unreliable as observed above. Further evidence will now be discussed with regard to the role of lysine as the first limiting amino acid in these diets.

Peaking time of individual essential amino acids in blood plasma (Table 9).

Because of the dependence of effective protein synthesis on the simultaneous supply of all needed amino acids at the sites of synthesis, differences in the relative peaking times of individual amino acids could

TABLE 11: PEAKING TIMES (h POSTPRANDIAL) OF INDIVIDUAL ESSENTIAL AMINO ACIDS OF PIGS.

Diet number		1	2	3	4	5	6	7	8	Grand Mean	Standard error of mean
Supplemental protein source(s)%	PNM	---	50	100	100	75	50	25	---		
	RSM	---	---	---	+	25	50	75	100		
	SBM	100	50	---	lysine	---	---	---	---		
Amino acids											
Arginine		2.5	2.4	1.7	2.2	1.7	1.8	1.6	0.8	1.84	0.31
Histidine		2.3	2.5	2.1	2.4	2.1	2.0	2.1	2.0	2.19	0.10
Isoleucine		1.8	2.4	1.5	2.3	2.4	1.7	1.0	0.6	1.72	0.42
Leucine		1.3	2.2	2.2	2.4	2.5	1.9	1.2	0.6	1.80	0.38
Lysine		1.8	1.6	0.5	1.4	0.5	0.8	0.6	0.5	0.96	0.31
Methionine		2.3	2.5	1.9	2.1	2.6	1.7	1.8	1.4	2.05	0.35
Phenylalanine		2.3	2.4	2.3	2.4	1.6	2.2	1.9	1.4	2.07	0.27
Threonine		2.3	2.4	1.2	1.5	1.4	1.9	1.7	1.0	1.68	0.48
Tryptophan		2.6	2.3	2.0	2.2	2.1	1.8	1.9	2.1	2.14	0.37
Valine		1.4	2.4	2.5	2.5	2.6	2.2	1.6	0.7	1.84	0.31

conceivably limit protein synthesis. However, there was no significant difference in peaking times, although the values ranged from 0.5 to 2.6 h. A consideration of the fact that blood plasma is a vehicle conveying amino acids and other nutrients to various sites of biological activities (synthesis and degradation) would lead one to question the validity of relying in this instance solely on conclusions indicated by normal statistical tests. It has been suggested (Pfander, 1969) that considerable protein synthesis occurs in a space of 30 min. Is it not logical, therefore, to suppose that any amino acid peaking out of rhythm with the rest could conceivably impede protein synthesis? It seems that the supply of essential amino acids to the sites of synthesis could be likened to the supply of raw materials to a factory. Apparent surpluses sometimes arising from unsynchronized deliveries of inputs may be discarded. A limiting input arriving a few minutes after the 'surpluses' have been eliminated may itself be rejected, due to lack of the other inputs that go into producing the goods of the factory.

In this instance, therefore, trends may provide a more meaningful picture of important biological differences than absolute quantitative differences. One noticeable trend with regard to peaking times of lysine in the various diets used in this study is that it takes longer for plasma lysine peaks to appear in animals fed the more efficient diets [Diets 1 (SBM 100), 2 (PNM 50, SBM 50), and 4 (PNM 100 + lysine)], compared with those fed the less efficient ones [Diets 3 (PNM 100), 5 (PNM 75, RSM 25), 6 (PNM 50, RSM 50), 8 (RSM 100) and 7 (PNM 25, SBM 75)]. On the average, plasma lysine peaks in animals fed the more efficient diets appeared 1.6 h postprandial, compared with 0.6 h in animals fed the less efficient diets, suggesting that the lysine in the less efficient diets was more readily released from their bound forms into the blood plasma. It is however, surprising that the supplemental lysine in Diet 4 (PNM 100 + lysine) apparently does not result in early peaking as would normally be expected, being more readily "available", where "availability" is defined as the rate at which the amino acid or any other nutrient is released from the diet for transportation to points of need in the body.

A comparison of the dietary amino acid concentrations (Table 2) with the postprandial plasma amino acid concentrations indicates some correlation between dietary lysine and plasma lysine levels. If at each peak concentration, the increase over the 0 h value is expressed as a ratio of the corresponding lysine level, the values 9.1, 7.7, 7.0, 4.9, 0.2, 4.6, 5.4, and 0.8 are obtained for Diets 1 (SBM 100), 2 (PNM 50, SBM 50), 4 (PNM 100 + lysine), 7 (PNM 25, RSM 75), 8 (RSM 100), 6 (PNM 50, RSM 50), 5 (PNM 75, RSM 25) and 3 (PNM 100), arranged in a descending order of nutritiveness. The inverse correlation between peaking times and growth rates has already been noted. In the more efficient diets, lysine is released relatively gradually to the tissues. In the less efficient diets, the lysine for protein synthesis is available for shorter periods (Figure 1). The peak is relatively sudden and high, so that, in fact, lysine degradation in that case might be high. Hagihira et al. (1961) in in vitro studies reported that lysine and the other basic amino acids, arginine, cystine and ornithine are actively transported across the epithelial cells of the small intestines. Furthermore, the maximal rate of transport of these amino acids were found to be 1/10 to 1/20 the rates for the transport of some of the neutral amino acids. They concluded that the small capacity for the transport of the basic amino acids explains the difficulty encountered by some investigators in obtaining significant

TABLE 12: PEAK CONCENTRATIONS (µm % POSTPRANDIAL) OF INDIVIDUAL ESSENTIAL AMINO ACIDS OF PIGS

Diet number		1	2	3	4	5	6	7	8	Grand Mean	Standard error of mean
Supplemental protein source(s)%	PNM	----	50	100	100	75	50	25	----		
	RSM	----	----	----	+	25	50	75	100		
	SBM	100	50	----	lysine	----	----	----	----		
Amino acids											
Arginine		22.1^{C}	29.7^{B}	28.8^{B}	37.6^{A}	30.3^{B}	31.5^{AB}	29.6^{B}	21.0^{C}	28.8	2.04
Histidine		12.8^{BC}	13.1^{BC}	11.2^{C}	12.3^{BC}	14.0^{B}	14.6^{AB}	16.6^{A}	16.5^{A}	13.9	0.70
Isoleucine		17.1	17.0	15.5	15.4	17.4	16.9	17.1	16.2	16.6	0.53
Leucine		24.1	22.4	18.4	20.6	22.6	21.8	22.7	22.0	21.8	1.14
Lysine		25.6^{AB}	18.1^{BC}	12.3^{C}	23.6^{AB}	17.4^{BC}	22.4^{AB}	27.3^{AB}	29.0^{A}	22.0	2.84
Methionine		4.9^{D}	5.2^{CD}	5.2^{CD}	5.8^{BC}	6.0^{ABC}	6.5^{AB}	7.0^{AB}	6.9^{A}	6.0	0.30
Phenylalanine		14.4^{CD}	18.0^{A}	16.5^{ABC}	16.0^{ABC}	16.9^{AB}	15.1^{BC}	14.8^{BC}	12.2^{D}	15.5	0.69
Threonine		20.6	18.7	18.8	18.0	27.7	24.6	21.4	18.4	21.0	3.50
Tryptophan		6.5	7.2	6.1	6.2	5.8	5.9	7.0	6.2	6.4	0.37
Valine		40.1^{A}	36.9^{AB}	29.9^{C}	33.8^{BC}	36.3^{AB}	35.6^{ABC}	38.2^{AB}	38.4^{AB}	36.1	1.74

net transport with high initial concentrations. It would appear that there is a similar and finite rate of utilization of lysine for protein synthesis. Consequently, in the present study, for those diets whose lysine is suddenly released into the blood plasma, at a rate far exceeding the synthetic capacity of the cells, the utilization rate for protein synthesis rather than the release or "inavailability" could be growth-limiting.

It is striking that the supplemental lysine in Diet 4 (PNM 100 + lysine) behaves like the bound lysine in SBM (Diet 1) or the bound lysine in SBM and PNM combined (Diet 2). These observations may not be as inconsistent as they appear, since there is evidence that during the first 2 h after injection of L-lysine-^{14}C, muscle constitutes a reserve of free lysine and that for the next 2 h, this reserve of lysine disappears to the benefit of other tissues (Arnal et al., 1971).

It would appear from this study that the lysine in RSM is quite readily released in digestion; but apparently the (muscle) storage of RSM lysine is not as effective as the muscle storage of the lysine in such protein supplements as SBM or PNM. One is led to postulate that there is something (some biochemical factor) in RSM which inhibits transient lysine storage when RSM-supplemented diets are fed to starting pigs. Added synthetic lysine in PNM-supplemented diets fed to starting pigs behaves like the bound lysine in SBM or PNM, because there is nothing to inhibit its storage. Therefore, as rapidly as it is released in digestion, it is avidly absorbed, stored in the muscle and released gradually for protein synthesis. Although there may be time lag of the order of 30 min. or more between the peak presentation of supplemental lysine or that of bound lysine in RSM, compared with the peak presentation of the bound lysine in SBM and PNM or the presentation of other amino acids for absorption, this is not as crucial as what happens after the lysine has been absorbed into the blood stream. In the case of diets supplemented predominantly with PNM, the problem is not so much that of inadequate release of bound lysine from the PNM nor of inability of the muscle to temporarily store the lysine. The problem is primarily that of low content of lysine in PNM. These observations are quite consistent with the superiority ranking of the diets: 1, 2, 4, 7, 8, 6, 5, and 3 (Appendix Table 1A and 1B). They could explain why supplementation of PNM with RSM promotes growth, but not as much as might be expected from the absolute increase in dietary lysine level brought about by the higher lysine content of RSM.

PIG EXPERIMENT, FINISHING PHASE, REPLICATES 1 AND 2

Days to Market, ADF, ADG, and EFC (Table 13)

Although statistical analyses were performed with and without adjustment for the variation in LW at allotment to the finishing diets, the conclusions were the same in either case; except that in some instances, the differences were more highly significant, when the weights at allotment were adjusted. For simplicity, therefore, the results presented here are based on adjusted weights.

In both replicates, diets significantly ($P \leq 0.05$) affected the ADG and the days from birth to 87 kg market weight. Pigs fed the PNM-supplemented diet gained more slowly ($P \leq 0.05$) and took longer ($P \leq 0.05$) to reach market weight than those fed the SBM-supplemented diet. For pigs 20 to 35 kg weight at allotment to the finishing diets, the lysine requirement is estimated to be 0.70% (NAS - NRC, 1968). The lysine content

TABLE 13: PIG EXPERIMENT, FINISHING PHASE: DAYS TO MARKET, AVERAGE DAILY FEED, AVERAGE DAILY GAIN AND EFFICIENCY OF FEED CONVERSION (REPS. 1 & 2, RESPECTIVELY).

Traits	Rep. no.	Treatment[+]		Sex		Breed[Δ]	
		SBM	PNM	Barrows	Gilts	Crossbred	Pietrain
Days to market	1	204.8	227.8**	213.8	219.7	209.9	223.0
	2	138.7	159.1*	146.3	157.5*	---	---
Average daily feed (kg)	1	2.25	2.03	2.28	1.99	2.11	1.93
	2	2.46*	2.19	2.48*	2.19	---	---
Average daily gain (kg)	1	0.66*	0.52	0.62	0.54	0.62	0.54
	2	0.74*	0.56	0.67*	0.57	---	---
kg feed/kg gain	1	3.39	3.93	3.70	3.67	3.43	3.55
	2	3.33	3.90	3.71	3.82	---	---

[+] SBM = Soybean meal-supplemented finishing diet.
PNM = Peanut meal-supplemented finishing diet.

[Δ] "Crossbred" means pigs sired by crossbred boars.
"Pietrain" means pigs sired by a Pietrain boar.

of the SBM-supplemented finishing diet was 0.66% i.e. 0.04% below the requirement. That of the PNM-supplemented diet was only 0.54% i.e. 0.16% less than the requirement. In both diets, the requirement of all other essential amino acids were either met or exceeded. In a well documented paper, Jones (1969) pointed out that in pigs, the growth rate, EFC, N retention and carcass quality are more closely related to the "available lysine" concentration of the diet than to the total lysine content. He added however, that once the imbalance of lysine, due either to a direct deficiency or to a low availability, has been corrected, further improvements in performance could be obtained by correcting the deficiencies of other amino acids. Based on this view and the known composition of lysine in the two finishers used in the present study, it is probable that differences in the dietary "available lysine" were responsible for the observed differences in the performance of the pigs fed the two finishing diets.

Although in both replicates, barrows grew faster and attained market weight sooner than gilts, in both traits (ADG and days to market), the difference was only significant ($P \leq 0.05$) in Replicate 2. Pietrain-sired pigs grew slower than crossbred-sired ones, and on the average took 14 days longer to reach market weight; but this difference was not significant. Part of the apparent difference could be associated with differences in the general thrift of the starting pigs, as there were fewer Pietrain-sired pigs than crossbred-sired pigs to choose from initially.

In Replicate 1, there were no significant treatment, sex or breed effects on ADF, although with the SBM-supplemented finishing diet, the barrows and the crossbred-sired pigs ate slightly more than their opposites.

In Replicate 2, significant difference ($P \leq 0.05$) in ADF was observed in favour of SBM-supplementation and of barrows. The apparent difference between Replicates 1 and 2 in the response to ADF may, in part, be due to breed differences in the two replicates. In Replicates 1 and 2, there were no significant differences in EFC. The trend of superiority was as noted for ADG, namely: SBM-supplementation was slightly better than PNM-supplementation, and crossbred-sired pigs better feed converters than their Pietrain-sired counterparts. Gilts appeared to be slightly better feed converters than barrows.

In summary, this study clearly indicates that as a finishing diet for pigs, a wheat-barley diet supplemented with solvent-extracted PNM and adequate in minerals and vitamins is inferior to the same basal diet supplemented with solvent-extracted SBM. The underlying factor appears ⑲ to be a relative insufficiency of available lysine in the PNM-supplemented diet.

Carcass analyses (Table 12).

Except for four slow-gaining pigs in Replicate 1 and three similar pigs in Replicate 2, which were marketed at less than 87 kg, the pigs were marketed at this weight or slightly heavier. Diet therefore, had no significant effect on market weight and carcass weight. However, because of the superior growth rate, the pigs fed the SBM-supplemented finisher tended to be slightly heavier because of narrowly missing the market weight the previous week and making substantial gains in the subsequent week. The percentage dressing loss in all animals decreases with increasing weight and age (McMeekan, 1940a); but as these animals were

TABLE 14: PIG EXPERIMENT, FINISHING PHASE: CARCASS ANALYSES.

Traits	Rep. no.	Treatment[+]		Sex		Breed[Δ]	
		SBM	PNM	Barrows	Gilts	Crossbred	Pietrain
Market weight kg	1	88.4	86.5	89.1	85.8	87.7	87.2
	2	88.7	86.2	87.6	87.2	--	--
Carcass weight kg	1	69.2	68.0	70.3	66.9	68.4	68.8
	2	69.4	67.0	68.7	67.7	--	--
Dressing %	1	78.0	78.5	78.7	77.9	77.9	78.6
	2	78.2	77.7	78.4	77.6	--	--
Length of side cm	1	73.9	73.4	73.4	74.0	74.1	73.2
	2	74.9	73.9	73.4	75.5*	--	--
Total backfat[▽▽] cm	1	9.91	10.29	10.72**	9.47	10.34	9.86
	2	10.11	10.07	10.90*	9.89	--	--
Area of loin cm^2	1	29.7**	26.4	28.0	28.1	26.5	29.7**
	2	29.7**	26.2	27.9	28.0	--	--
Lean in ham face %	1	48.0	47.4	46.4	49.0*	45.4	50.0**
	2	51.4	50.8	49.1	53.1*	--	--
Ham wt./carcass wt. %	1	27.7	27.7	27.1	28.3**	27.7	27.6
	2	27.3	26.9	27.1	27.2	--	--
Grade index	1	101.6	99.6	100.2	100.9	99.3	101.8*
	2	101.1**	97.1	98.4	99.8	--	--
Canadian ROP[ϕ] index	1	68.8	67.6	68.0	68.3	66.9	69.5**
	2	69.5	68.1	69.1	68.4	--	--

[+] SBM = soybean meal-supplemented finishing diet; PNM = peanut meal-supplemented finishing diet.

[Δ] "Crossbred": pigs sired by crossbred boars, "Pietrain": pigs sired by a Pietrain boar.

[▽▽] Sum of three measurements (shoulder, loin and back).

[ϕ] Canadian Record of Performance.

marketed at practically identical average weights between treatments, treatment did not significantly influence dressing percentage.

The length of carcasses was also similar, regardless of the finishing diets fed; although the pigs consuming the SBM-supplemented finisher were slightly longer, reflecting their slightly heavier weight. Compared with the development of depth and breadth, length is an early maturing trait (McMeekan, 1940a), and therefore, less likely to be affected by nutritional input imposed at a later stage in life, having as it does a relatively higher competitive efficiency for nutrients early in life.

Although the treatment difference in backfat thickness was not significant, the pigs fed the PNM-supplemented finisher tended to be fatter. In loin area, in both replicates, there was a significant difference ($P \leq 0.01$) in favour of pigs fed the SBM-supplemented finisher. This is consistent with the expected interaction of a varied nutritional environment with the law of "developmental direction". The latter asserts that there is in all organic forms, differential growth of tissues (bone, muscle and fat in that order), and that the trend consists of an anterior-posterior directional development, with limbs behaving as relatively earlier developing part: hind limbs slightly later developing than fore limbs. This concept of allometric growth, which has been validated by more recent studies (Butterfield and Berg, 1966a; Richmond and Berg, 1971a, 1971b), claims that greater variability is associated with a later developing tissue, such as loin area, than with an earlier developing tissue, such as the brain (McMeekan, 1940a). Hence, the observed significant difference in loin area due to superimposed nutritional environments.

In both replicates, the pigs were allotted to the finishing diets at an average weight of 21 - 25 kg. Therefore, their amino acid requirements would be those recommended (NAS - NRC, 1968) for 20 - 30 kg pigs. A comparison of the amino acid analyses (Table 2) of the two finishing diets with the nutrient requirement indicates that the two diets are adequate in all essential amino acids, except lysine, in which the SBM-supplemented finisher is superior to the PNM-supplemented finisher. Lysine insufficiency in the latter could therefore limit protein synthesis relative to that obtained in pigs fed the alternative diet; for amino acid deficiencies are quite critical between 34 and 46 kg liveweight, when the growth of lean tissue is at its maximum in relation to total daily food intake (Fowler, 1966).

Evidence of a restricted protein synthesis is supplied by the comparatively poorer EFC of the pigs fed the PNM-supplemented finisher. The EFC of lean tissue is approximately 2 kg feed/kg gain; that of fat alone is 6 to 7 kg feed/kg gain. Generally speaking, therefore, the better the EFC, the leaner the carcass (Fowler, 1966). The two finishers were isocaloric and met NAS - NRC (1968) requirement for digestible energy. With lysine deficiency limiting the fat-free tissue growth in the PNM-supplemented finisher more than that in the SBM-supplemented finisher, the energy in the former diet in excess of requirements for maintenance and fat-free body growth would be deposited as body lipid, especially as the animal approaches mature fat-free size (ARC, 1967). It would appear that for pigs fed a PNM-supplemented diet, a reduction of dietary energy (to an optimum ascertainable by experimentation) could be of practical benefit where lean carcasses are desired.

The lean in ham face, the ham weight/carcass weight percentages and the ROP index show the same trends as the above, the slight differences being in favour of the pigs fed the SBM-supplemented diet. In grade index, the most important economic criterion, however, the difference in favour of the SBM-supplemented finisher was significant ($P \leq 0.01$) in Replicate 2. Lack of significance in Replicate 1 might be due to the relatively superior lean growth of the Pietrain-sired pigs featured in that replicate.

Barrows had more ($P \leq 0.05$) backfat and gilts a higher ($P \leq 0.05$) lean in ham percentage. These observations are consistent with the significantly higher ($P \leq 0.05$) N retention recorded for gilts in this study.

In carcass length and ham weight/carcass weight percentage, sex effects differed between replicates. In Replicate 2 but not in 1, the carcasses of gilts were significantly ($P \leq 0.05$) longer than those of barrows. In Replicate 1 but not in 2, gilts had a significantly higher ham weight/carcass weight percentage. Since Pietrain-sired pigs were included in Replicate 1 but not in 2, the observed differences might be associated with breed effects. Because of the design of the experiment, it was difficult to check on the interaction between sex and breed.

Pietrain-sired pigs had slightly less carcass fat, a significantly larger loin area ($P \leq 0.01$), a higher ($P \leq 0.01$) lean in ham face percentage, a higher ($P \leq 0.05$) grade index and a superior ($P \leq 0.01$) ROP index. These breed-dependent effects demonstrate the well known existence of hereditary differences between breeds of the same species. It is this kind of difference that led Blaxter (1972) to caution that when considering animal experiments, we may well have to qualify our statements and recognize that we cannot readily draw conclusions about "the rat" or "the pig". We must be apprehensive of the fact that our findings might only apply to a particular population.

The two finishing diets used in this study were superimposed on pigs which were not only fed starting diets differing widely in nutritive value, but also conceivably differing in preweaning nutritional environment, coming probably from different suckling positions in the udder and from dams with varied milk yields. The fact that there were relatively few significant differences in the carcasses as a whole, demonstrates among other things the remarkable recuperative capacity of pig tissues, a fact brought out by McMeekan (1940c).

RAT EXPERIMENT, REPLICATES 1 AND 2.

Gain and food intake (Table 15) for a 6-week test period.

The highest average gain (188.1 g) for a 6-week test period was made by rats fed the control SBM-supplemented diet. This was not significantly different ($P \leq 0.01$) from those of rats fed Diets 2 (PNM 50, SBM 50), 3 (PNM 100), 4 (PNM 100 + lysine) and 9 (PNM 50, SBM 50 + lysine), which averaged 184.6, 180.1, 184.9 and 164.8 g respectively. It was surprising that statistically, rats receiving Diet 3 (PNM 100) with a dietary lysine content of 0.65% (Table 2) compared with an estimated requirement of 0.90% (NAS - NRC, 1972) fared as well as those on the control diet containing 0.92% dietary lysine. A possible explanation could be that the lysine requirement of the rats used in this study was below 0.90%. This view is supported by the fact that supplementation of Diet 3 (PNM 100) with lysine to raise the dietary lysine level from 0.65 to 0.76% did not significantly increase gain.

The PNM used in this study was the same as in a previous study (Bowland - Orok, private communication, 1973), in which the results suggested that in PNM-supplemented diets, methionine, lysine and possibly threonine may be limiting for the rat. The methionine-cystine content of the ten diets used in the present study ranged from 0.52 - 0.67%. A comparison of these values with the requirement of 0.60% (NAS - NRC, 1972) suggests that on the whole, the methionine-cystine requirement was only marginally met and could be limiting. This may provide an alternative and more likely explanation for the observed similarity in response between rats fed Diet 1 (SBM 100) and those on Diets 2 (PNM 50, SBM 50) and 3 (PNM 100), and for lack of response to lysine supplementation of

TABLE 15: INFLUENCE OF DIETS AND SEX ON FOOD CONSUMPTION (FC), GAIN AND EFFICIENCY OF FOOD CONVERSION (EFC) OF RATS, 6 WEEKS ON TEST

Diet no.	Supplemental protein source(s)%	FC g	Gain g	EFC g food /g gain
1	SBM 100	627.6^{a}	188.1^{a}	3.44^{a}
2	PNM 50, SBM 50	621.1^{ab}	184.6^{ab}	3.49^{a}
3	PNM 100	632.8^{a}	180.1^{ab}	3.64^{ab}
4	PNM 100 + lysine	624.6^{a}	184.9^{ab}	3.51^{a}
5	PNM 75, RSM 25	584.3^{bc}	158.4^{bc}	3.82^{abc}
6	PNM 50, RSM 50	545.8^{bcd}	144.1^{cde}	4.02^{bc}
7	PNM 25, RSM 75	514.2^{de}	130.4^{de}	4.19^{c}
8	RSM 100	475.8^{e}	122.2^{e}	4.07^{bc}
9	PNM 50, SBM 50 + lysine	594.4^{bc}	164.8^{abc}	3.73^{abc}
10	RSM 100 + lysine	545.2^{cd}	151.2^{cd}	3.78^{abc}
Sex				
	Male	655.7^{**}	203.1^{**}	3.28^{**}
	Female	497.5	188.7	4.26
Grand mean		576.6	160.9	3.77
Standard error of mean		16.8	6.4	0.11

Diet 2 (PNM 50, SBM 50) and 3 (PNM 100). Simultaneous supplementation of methionine and lysine would be needed to elicit favourable responses in PNM-containing diets, if they were equally limiting in methionine and lysine. This is further supported by the observation that the rats fed the control diet had an average gain of 188.1 g for a 6-week period or an average gain of 31.4 g per week, which is somewhat suboptimal for rats from 3 to 9 weeks of age.

The amino acid compositions of Diet 3 (PNM 100) and 5 (PNM 75, RSM 25) were almost identical, except in valine, phenylalanine-tyrosine, leucine and arginine in all of which Diet 5 (PNM 75, RSM 25) was slightly lower, yet met NAS - NRC (1972) requirements for rats. It was surprising, therefore, that rats fed the latter diet grew significantly ($P \leq 0.01$) less than those fed Diet 3 (PNM 100). Food intake for rats fed Diet 5 (PNM 75, RSM 25) was also significantly ($P \leq 0.01$) less than those on Diet 3 (PNM 100). On the basis of the apparent similarity in dietary amino acid composition, it would appear that the 0.65% lysine in Diet 5 (PNM 75, RSM 25) was not as useful as the 0.65% lysine in Diet 3 (PNM 100). The corollary is that the replacement of PNM with 25% RSM while not affecting the absolute amount of lysine in the diet, appeared to lower the amount of lysine available for protein synthesis. This association of a reduced gain with the substitution of prepress solvent-extracted RSM for 25% of solvent-extracted PNM lends additional support to the view derived from plasma amino acid studies with pigs (page 64) that there appears to be something in RSM which inhibits transient muscle storage of lysine, thereby reducing it's metabolic usefulness. Lysine supplementation of Diet 8 (RSM 100) to raise the level from 0.88 to 1.06% significantly ($P \leq 0.01$) improved both food consumption (FC) and gain, but this enhanced performance was not nearly as good as that achieved with Diet 1 (SBM 100); although the latter contained less (0.92%) lysine. This again demonstrates the fact that the lysine in RSM is apparently metabolically less useful compared, for example, with the lysine in SBM or PNM.

Kumta and Harper (1961) reported an almost immediate decrease in the FC of young rats fed on amino acid deficient diet. They observed that the FC and hence growth of rats receiving such imbalanced diet were stimulated when the concentrations of the most limiting amino acids or the quantity of balanced protein in the diet were increased. Their observations agree with the findings of the present study and strengthen the above evidence that the lysine in RSM used in this study was not as metabolically useful as that in the PNM.

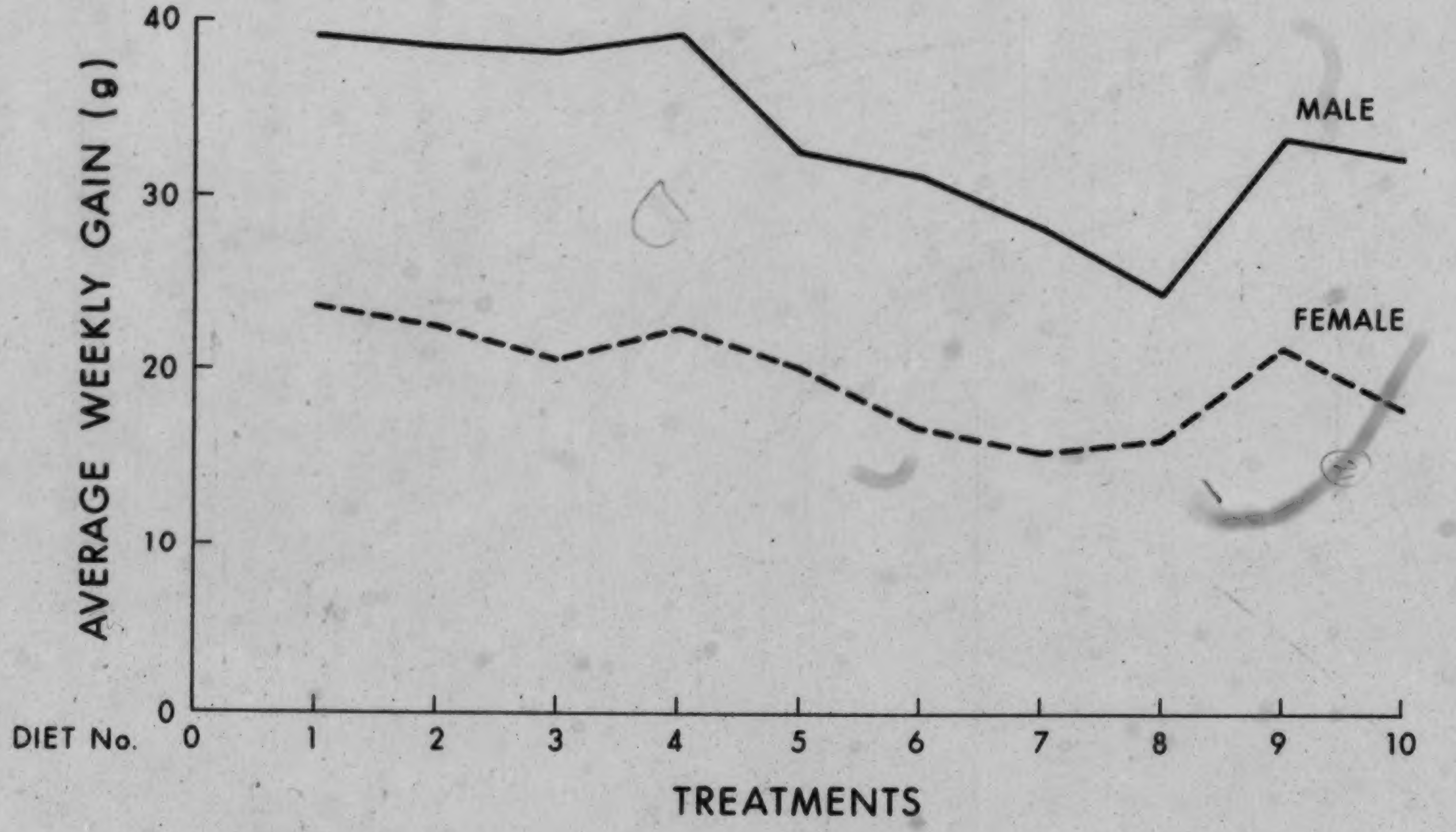
Increased substitution of RSM for PNM, Diets 6 (PNM 50, RSM 50) and 7 (PNM 25, RSM 75), consistently decreased both food intake and growth, so that the lowest FC and growth were obtained when RSM fully replaced PNM. However, the growth of rats fed Diets 6 (PNM 50, RSM 50), 7 (PNM 25, RSM 75) and 8 (RSM 100) were not significantly different. The same was true of FC, except that the FC of rats fed Diet 8 (RSM 100) was significantly less ($P \leq 0.01$) than that of rats fed Diet 6 (PNM 50, RSM 50). It is possible that at higher levels of RSM substitution, not only lysine but also goitrogens are involved. Lo and Hill (1971) with rats observed that a high intake of glucosinolates affected thyroid function by blocking iodine trapping and iodine organification, whereas a low intake of glucosinolate stimulated thyroidal iodine uptake and slowed down iodine release rate. Because of the known influence of the thyroid gland on metabolic rate, it follows that the modification of thyroid function by high dietary levels of RSM can have a corresponding negative influence on food utilization and on gain.

EFC was similar for rats on all diets, with no significant difference between five diets, 1 (SBM 100), 2 (PNM 50, SBM 50), 3 (PNM 100), 4 (PNM 100 + lysine), 5 (PNM 75, RSM 25) and the last two diets, 9 (PNM 50, SBM 50 + lysine) and 10 (RSM 100 + lysine). The poorest EFC ($P \leq 0.01$) was observed in rats fed Diet 7 (PNM 25, RSM 75); but this was not significantly different from those of rats fed Diet 8 (RSM 100), 6 (PNM 50, RSM 50), 5 (PNM 75, RSM 25), 9 (PNM 50, SBM 50 + lysine) and 10 (RSM 100 + lysine). This rather unique statistical consistency in the EFC of rats fed diets adequately supplemented with minerals and vitamins but containing various percentages of RSM is again suggestive that apart from amino acid insufficiency, the growth inhibitors in rapeseed (Astwood et al., 1949a;Ballester et al., 1973; Joseffson et al., 1972) may also be involved. Histopathological examination of the rat thyroids, for example, showed increased distortion of thyroid tissues (Figures 5a - 6b) with increasing levels of RSM; although diets had no significant effects on thyroid weights. These Figures are discussed on page 89 . Although goitrogenecity has been reported for soybean and peanuts (McCarrison, 1933), the effect is usually not manifest in the thyroid gland of rats fed well processed PNM- or SBM-supplemented diets and adequately supplemented with minerals and vitamins (McCarrison, 1933).

The fact that amino acid imbalance (Kumta et al., 1961), goitrogens (Ballester et al., 1973), unidentified factors (Josefsson and Munck, 1972), which are associated with rapeseed may inhibit FC and growth and impair EFC make it difficult to pinpoint a single factor in any observed impairment of FC, growth, and EFC in animals fed RSM-supplemented diets. Consequently, from the evidence of the present study, one can only say that the relatively low content of lysine in RSM compared with the amount in SBM, together with its lower metabolic availability compared, for example, with the lysine in PNM, is one of the many factors that could contribute to the impaired FC, growth and EFC in rats fed diets supplemented with RSM.

Sex significantly affected ($P \leq 0.01$) FC, growth and EFC. This was true for all diets (Figures 2 - 4). However, the wide gap in EFC in both Diets 5 (PNM 75, RSM 25) and 7 (PNM 25, RSM 75) can neither be explained solely on the basis of amino acids, nor on a simple direct relationship of intake of goitrogens to thyroid function; for otherwise Diets 8 (RSM 100) and 7 (PNM 25, RSM 75) would show similar response patterns.

Bowland and Standish (1966) reported a poorer EFC for female rats receiving RSM-supplemented diets compared with those fed a SBM-supplemented basal diet. They noted that RSM depressed the EFC of male rats for the first 2 weeks on test compared with that of male rats fed the basal diet. For the rest of the 11-week test period, there were no significant differences in EFC between male rats fed RSM-supplemented diet and those on the control. In the present study, the increased superiority of males over females in EFC was associated with both age and increasing dietary levels of RSM. For example, the EFC data for Diets 5 (PNM 75, RSM 25), 6 (PNM 50, RSM 50), 7 (PNM 25, RSM 75) , 8 (RSM 100) and 10 (RSM 100 + lysine) indicated that whereas males consumed an average of 3.01 g food/g gain during the first 3 weeks on test, they consumed 4.11 g food/g gain in the subsequent 3 weeks. This represents approximately a 33% increase in food requirement per g gain. The corresponding figure for female rats were 3.45 g food/g gain during the first 3 weeks on test, 6.92 g food/g gain in the subsequent 3 weeks: approximately 100% increase



PROTEIN SOURCE										
SBM	100	50	-	-	-	-	-	-	50	-
PNM	-	50	100	100	75	50	25	-	50	-
RSM	-	-	-	+ lysine	25	50	75	100	+ lysine	100 + lysine

Figure 2: Average weekly gain per treatment per sex.

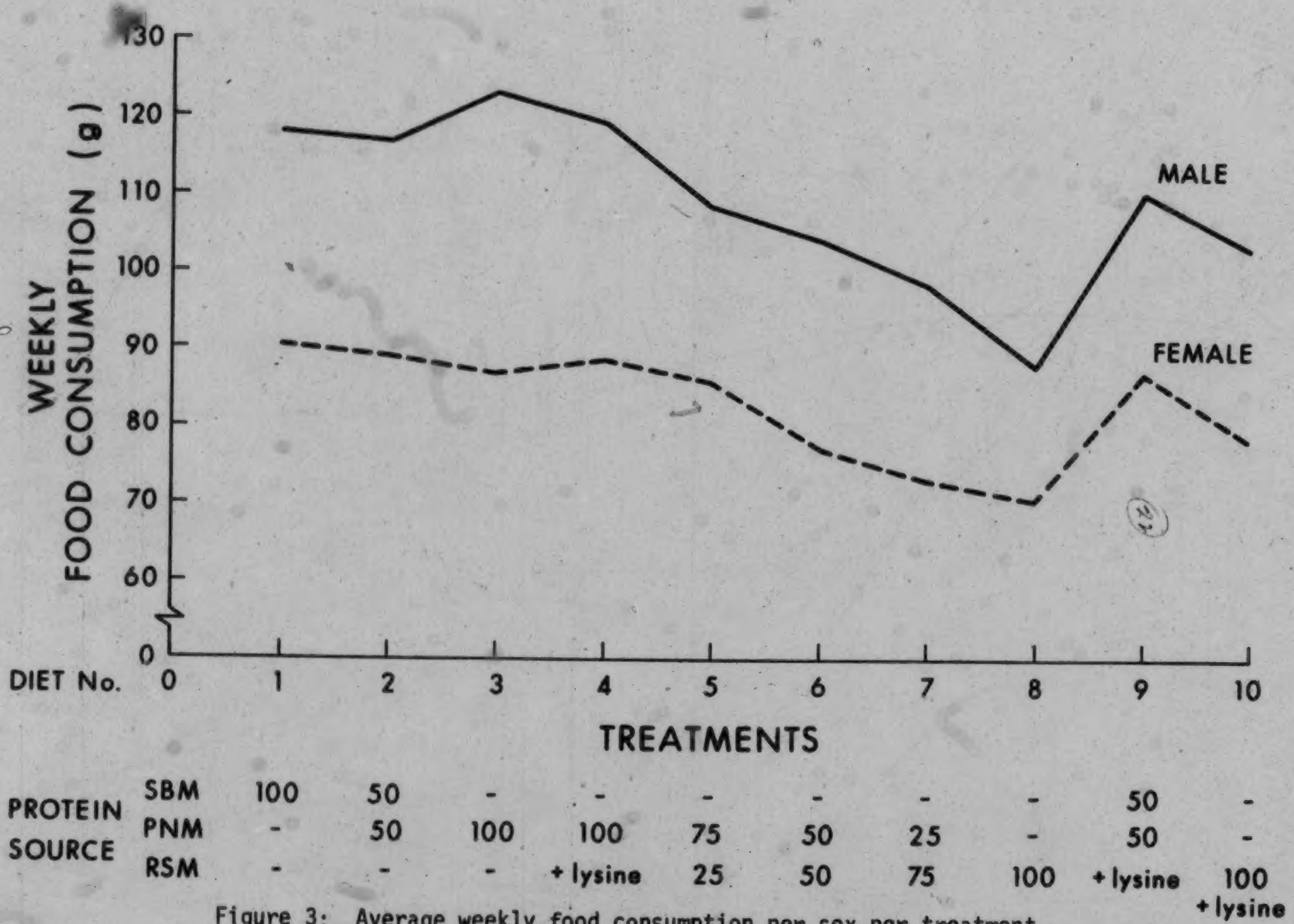


Figure 3: Average weekly food consumption per sex per treatment.

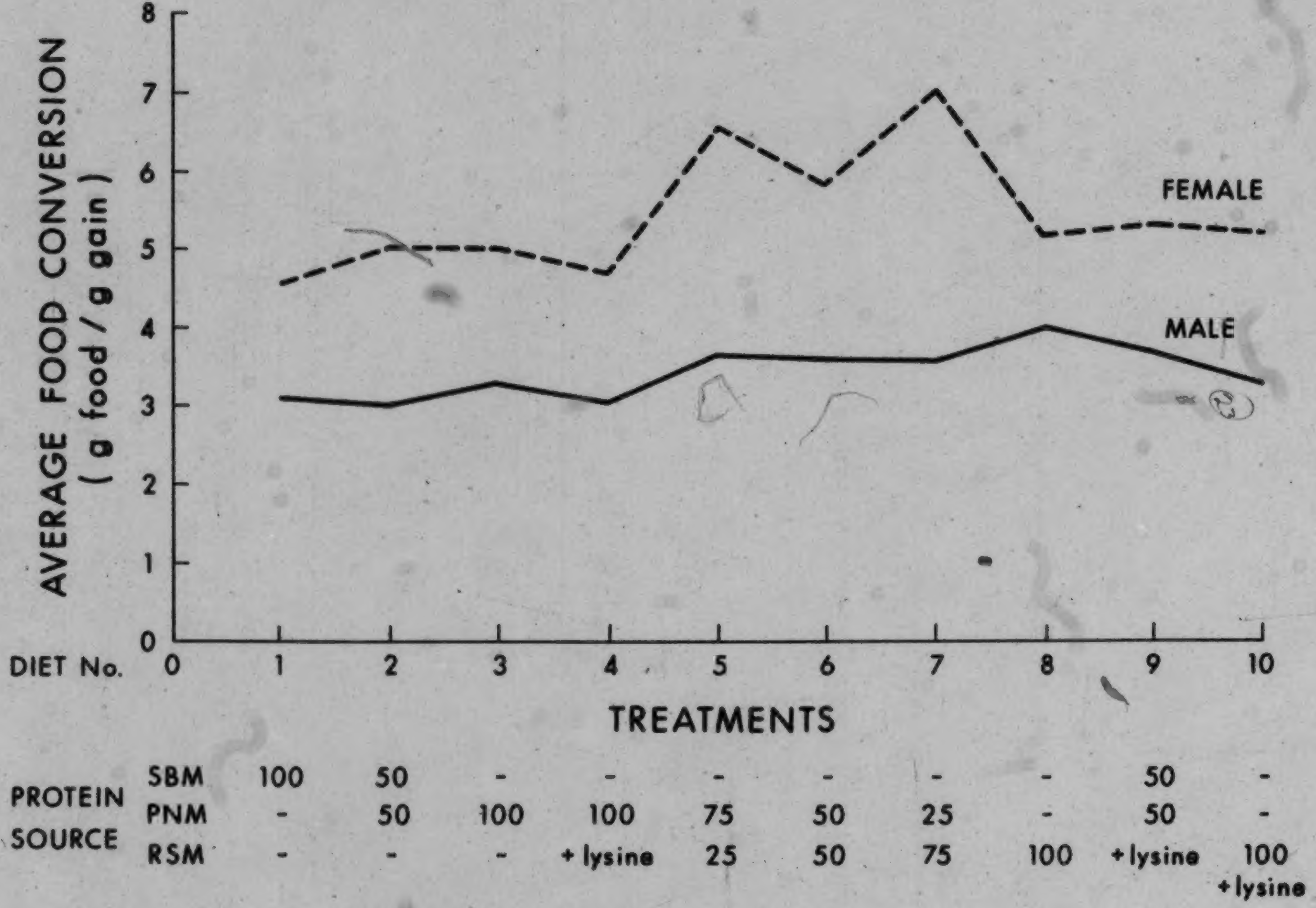


Figure 4: Average food conversion per sex per treatment.

in the food requirement per g gain. For the five RSM-supplemented diets from which these data were drawn, it can therefore be said that during the first 3 weeks on test, female rats consumed 14.6% more food per g liveweight gain than male rats and that in the subsequent 3 weeks this relative increase in food intake was 68.4%. For the rats fed the control diet, the corresponding average increase in the g food/g gain of females relative to males was 13.5% from weeks 1 - 3 and 83.7% from weeks 4 - 6. Starting with Diet 5 in which 25% of the PNM was replaced by RSM to Diet 10, in which RSM completely replaced PNM, the relative percentage increase in the g food/g gain required by females compared with males were 89.4, 51.1, 108.0, 24.1% and 75.9 respectively.

The reduced gap in EFC between males and females when PNM is fully replaced by RSM probably indicates that the threshold of tolerance to dietary RSM has been exceeded in both sexes. Addition of synthetic lysine in Diet 10 (RSM 100 + lysine) appeared to improve the EFC of male rats only and thus widened the percentage difference in the g food/g gain due to sex from 24.1 in Diet 8 (RSM 100) to 75.9 in Diet 10 (RSM 100 + lysine). Apparently, male and female rats differ in their lysine requirements. This study indicates that the EFC's of male and female rats respectively ㉔ are impoverished by dietary RSM. Secondly, the data (Figure 4) seem to suggest that when the EFC is used as the criterion of comparison, both male and female rats have intermediate optima in their tolerance to dietary RSM. This concept of the existence of an intermediate optimum in the adaptation of animals of different genotypes and sexes to a continuous array of bivariate (or multivariate) nutritional environments has been reported for mice (Bateman, 1971). The EFC data (Figure 4), particularly for the female rats, seem to indicate that when graded levels of dietary

RSM are fed to rats, the best EFC is obtained at an intermediate level of RSM-supplementation. It would also appear (Figure 4) that the threshold of tolerance of male rats to dietary RSM is higher than that of female rats, an observation which justifies the frequent recommendation that caution be exercised in the level of RSM included in diets, particularly those intended for female animals.

Although Lo and Hill (1971) suggested that a high intake of glucosinolates affects thyroid function by blocking iodine trapping and iodine organification, whereas a low intake of glucosinolates stimulates iodine uptake and slows down iodine release rate, they did not specify the absolute intake of glucosinolates at which a change in thyroid response would be expected to occur. Implicit in their study is the fact that the response of rat thyroid to glucosinolate intake is not linear. Differential sex reaction to non-linear thyroid response to glucosinolate intake may therefore account for some of the observed apparent sex differences in the EFC of rats consuming diets supplemented with different levels of RSM.

In these studies, starting pigs and weanling rats were subjected to the same dietary treatments, except that the rats had two additional diets, namely, Diets 9 (PNM 50, SBM 50 + lysine) and 10 (RSM 100 + lysine). This approach was based on the view well put by Blaxter (1972) that in the development of the framework of principles in nutrition, the final test of the generality can only come through experiments and observations with a wide variety of living things.

This study reveals striking differences between Sprague-Dawley rats, The University of Alberta strain and pigs, the breeding of which was specified earlier. Whereas for pigs, the addition of synthetic lysine to the PNM-supplemented diet considerably though not significantly increased feed intake, with a concomitant improvement in ADG and in EFC, rats did not respond positively to the addition of synthetic lysine to the PNM-supplemented diet. With pigs, SBM was the best protein supplement followed by RSM, with PNM ranking as third, when feed intake, gain and the kg feed/kg gain were used as the criteria of comparison. On the other hand, when the same diets and evaluation criteria were used with rats as the test species, PNM proved to be as good a protein supplement as SBM. With pigs, increasing substitution of RSM for PNM tended to improve feed intake, ADG and EFC; whereas with rats, the opposite trend was observed.

In feed intake, ADG and EFC, the only significant difference ($P \leq 0.05$) between barrows and gilts was in EFC, and this was in favour of gilts. In food intake, gain and EFC, male (non-castrated) rats were superior ($P \leq 0.01$) to female rats. Rats responded positively ($P \leq 0.01$) to the addition of synthetic lysine to the RSM-supplemented diet in food consumption and gain but not in the EFC. The potential response of pigs to the addition of synthetic lysine to the Span RSM was not assessed in this study, due to insufficient Span RSM, but a concurrent study (Bowland and Briggs, 1973, unpublished data) indicated that pigs will generally not respond to the addition of synthetic lysine to wheat and barley-based diets supplemented with Span RSM.

The observed species differences in the response to identical dietary treatments support the view (Blaxter, 1972), that although we are slowly building up a fabric of principles in nutrition in which we can begin to distinguish an underlying unity of nutritional response in biological systems, nutritional tests with one animal species provide little more than a tenuous guide or no guide at all for other species, "because the responses of one species to dietary insult are not (necessarily) the same as those of another".

Thyroid weights and thyroid histology.

Sex, but not diets, significantly ($P \leq 0.01$) influenced thyroid weights, with the thyroid weight of female rats averaging 6.3 mg/100 g liveweight compared with 4.9 mg/100 g for males. Levine et al. (1933) found no sex difference in the thyroid weights of young rats exposed to goitrogenic diets. However, with sexually mature rats, they observed a significant sex difference, with the thyroid weights, of female rats averaging 10.9 ± 0.23 mg/100 g body weight, compared with 9.6 ± 0.24 mg/100 body weight for males. The thyroid weights of rats on their control diet averaged 12.6 ± 0.19 (range, 10.5 - 16.0) mg/100 g body weight. The corresponding figures for rats fed their goitrous diets was 53.2 ± 0.92 (range, 24.1 - 126.0) mg/100 g body weight: a fourfold enlargement over those of rats fed normal diet. The thyroid weights obtained in the present study are smaller than those reported by Levine et al. (1933). The relatively low level of goitrogens in Span RSM, the refinement in processing technique, and adequate supplementation of the basal diet with minerals and vitamins may partially account for the differences.

Histological examination showed various degrees of hyperplastic goiter, depending on the dietary protein source (Figures 5a - 6b). The most goitrogenic protein supplement was RSM, followed by SBM, followed by PNM. Consequently, the rank order of the diets based on the severity of histopathological lesions was Diets 8 (RSM 100), 10 (RSM 100 + lysine), 7 (RSM 75, PNM 25), 6 (PNM 50, RSM 50), 5 (PNM 75, RSM 25), 2 (PNM 50, SBM

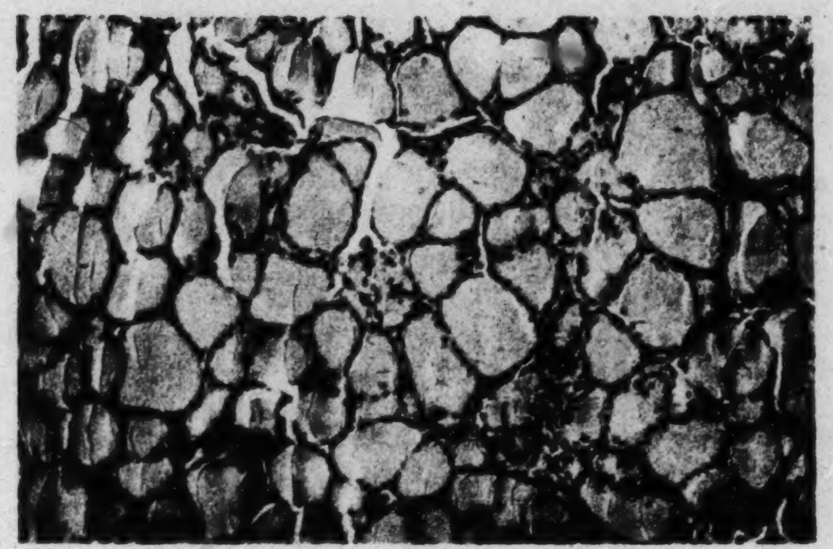


Figure 5(a): Rat thyroid photomicrograph of a male rat after 6 weeks on diet 1 (SBM-supplemented). Thyroid weight 5.8 mg/100 g body weight. Follicles uniform in size and uniformly stained, most with colloid.

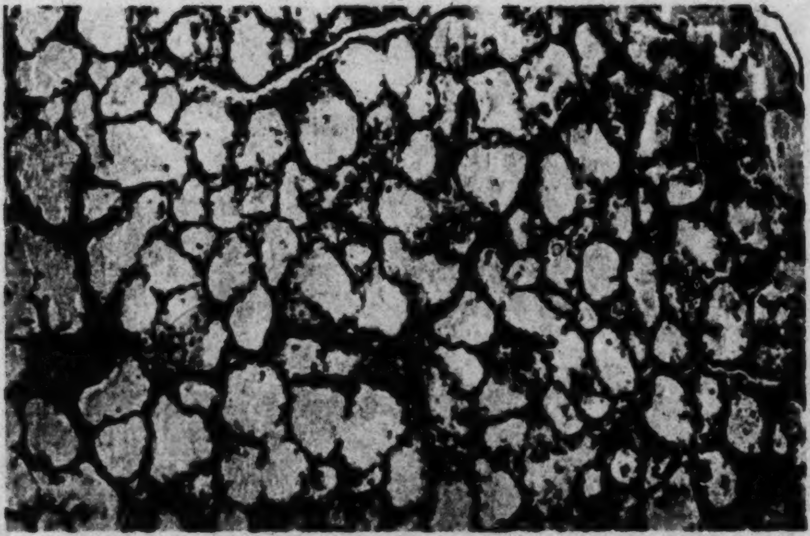


Figure 5(b): Rat thyroid photomicrograph of a female rat after 6 weeks on diet 3 (PNM-supplemented). Thyroid weight 6.9 mg/100 g body weight. Follicles uniform in size and uniformly stained, most with colloid.

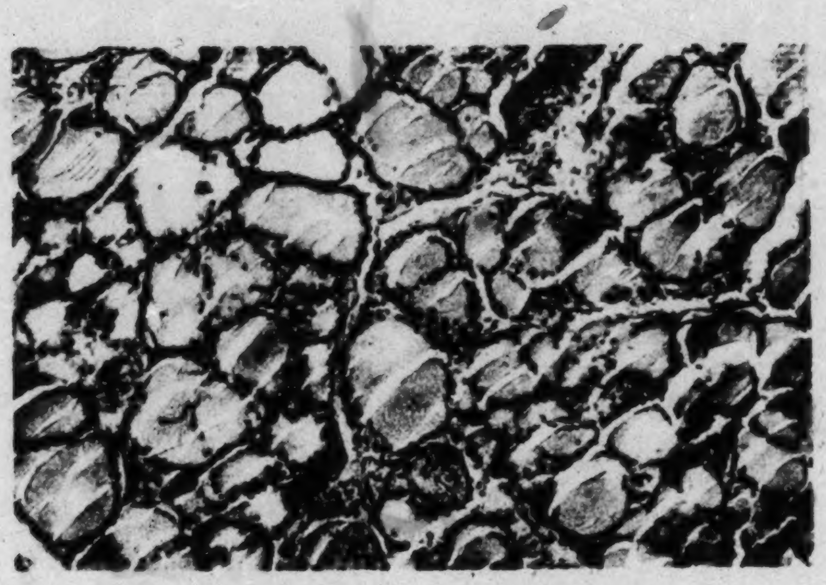


Figure 6(a): Rat thyroid photomicrograph of a female rat after 6 weeks on diet 9 (supplemented with 50% PNM and 50% SBM). Thyroid weight 5.1 mg/100 g body weight. Most follicles contain colloid, but there is more variation in follicular size and colloid staining than in Figures 5(a) and 5(b).

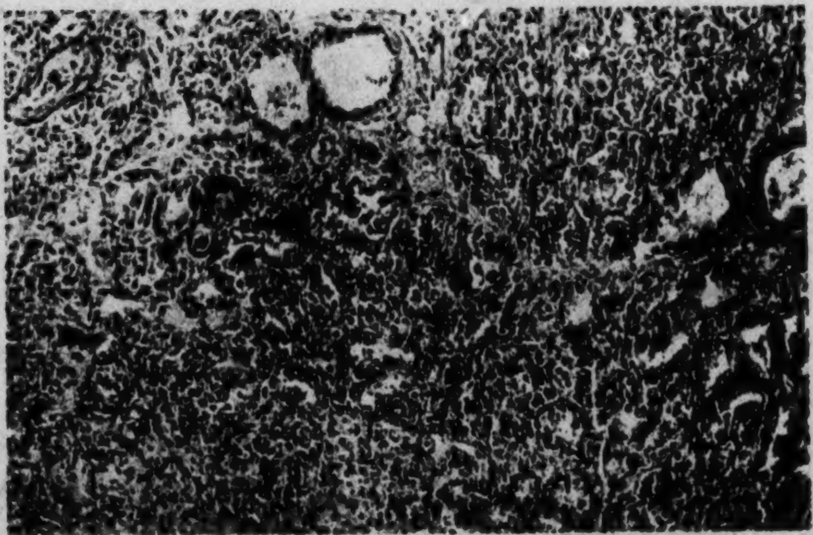


Figure 6(b): Rat thyroid photomicrograph of a male rat after 6 weeks on diet 10 (RSM & lysine-supplemented). Thyroid weight 5.0 mg/100 g body weight. Only a few follicles contain colloid. Colloid is pale-stained. Epithelia are hyperplastic.

50), 9 (PNM 50, SBM 50 + lysine), 1 (SBM 100), 4 (PNM 100 + lysine) and 3 (PNM 100). The most normal thyroid sections had a homogeneous, well-stained colloid in most of the follicles; although a few of the follicles contained no colloid. The follicles themselves were relatively uniform in size. In sections of thyroid glands suffering from severe goiter (e.g. those of rats fed RSM 100 ± lysine), only a few peripheral follicles contained pale-staining colloid. The remaining colloid - depleted follicles had hyperplastic epithelia sloughed into them. There were also focal accumulations of nuclear debris indicative of advancing "exhaustion atrophy"[1]. In between these two extremes were thyroid glands of varying degrees of thyrotoxicity, as evident from the extent of thyroid tissue distortion. Variations between individual animals within diets were slight.

Whereas, gravimetrically, it was not possible to detect significant differences between diets in goitrogenecity, histologically, there were substantial dietary differences. Wilgus _et al_. (1953), after extensive studies of the iodine requirements of chickens concluded that the histological evidence of goiter was more critical than thyroid weight and that the estimation of thyroid status based on gland weight does not give reliable evidence of the actual functional status of that gland. The present study is in complete accord. According to Wilgus _et al_. (1953), esterone and testosterone have a marked effect on thyroid glands of mature rats fed goitrous diets. It would therefore appear from this that the observed sex differences in the thyroid response of sexually - maturing rats to goitrous diets have an underlying genetic and biochemical bases.

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[1] As characterized in Modified Mallory's triple connective tissue stain (Wilgus _et al_., 1953).

Rat carcass composition (Table 16).

Empty body weight (EBW) and EBW EFC.

The gains in EBW ranged from 125.8 - 168.7 g. Six diets [namely, 3 (PNM 100), 4 (PNM 100 + lysine), 5 (PNM 75, RSM 25), 6 (PNM 50, RSM 50), 7 (PNM 25, RSM 75) and 9 (PNM 50, SBM 50 + lysine)] had the same influence statistically ($P \leq 0.01$) on EBW gain. There was successive overlapping between the various statistical groups of treatments.

Similar trends were observed in EBW percentage and EBW EFC, with more overlapping between the various groups of statistically identical treatments. In a previous study (Orok and Bowland, 1973, unpublished data) with Canadian SBM and Nigerian PNM as protein supplements and with Canadian yellow corn, Nigerian yellow corn and guinea corn as sources of energy, the EBW percentages of rats were not significantly different between diets. In another study (Orok and Bowland, 1973, unpublished data) with PNM and SBM as protein supplements and yellow corn, cassava meal and cocoa husks as energy sources, diets had some significant effects on EBW percentages, thus suggesting in conjunction with the previous findings that depending on the kind of diets compared, EBW percentages may or may not differ significantly. The present study with a few significant differences and extensive overlapping between groups of statistically different treatments lead to similar conclusion. SBM-supplemented diets tended to yield the highest EBW percentage, followed by PNM-supplementation, followed by RSM-supplementation. This order of superiority was true not only of EBW gain, but also of EBW expressed as a percentage of LW and of EBW - EFC. Lysine-supplementation in Diets 4 (PNM 100 + lysine), 9 (PNM 50, SBM 50

TABLE 16: INFLUENCE OF DIETS AND SEX ON RAT CARCASS COMPOSITION[1]

Diet no.	Supplemental protein source(s) %	EBW gain g	EBW /LW %	EBW EFC	DM gain %	DM/ EBW %	DM EFC	Carcass composition (DM basis) Protein %	Fat %	Ash %	Energy (kcal) stored in: Carcass protein	Carcass fat	Total Carcass
1	SBM 100	168.6^{a}	92.8^{a}	3.70^{a}	62.6^{a}	34.9^{a}	10.00^{a}	58.8^{bc}	30.4^{a}	10.1^{b}	252.16^{ab}	216.76^{a}	468.91^{a}
2	PNM 50, SBM 50	168.7^{a}	92.1^{abc}	3.76^{ab}	61.0^{a}	34.0^{ab}	10.46^{ab}	62.3^{abc}	26.4^{ab}	10.7^{ab}	261.75^{a}	183.62^{abcd}	445.38^{ab}
3	PNM 100	160.8^{ab}	92.3^{ab}	4.03^{abc}	59.0^{ab}	34.4^{ab}	11.17^{ab}	58.4^{c}	30.2^{ab}	10.6^{ab}	240.68^{abcd}	210.31^{ab}	450.99^{a}
4	PNM 100 + lysine	161.1^{ab}	91.9^{abc}	3.97^{abc}	59.4^{ab}	34.3^{ab}	11.02^{ab}	61.0^{abc}	28.4^{ab}	10.5^{ab}	247.40^{abc}	196.41^{ab}	443.80^{ab}
5	PNM 75, RSM 25	147.3^{abcd}	92.1^{abc}	4.27^{c}	53.3^{abcd}	34.1^{ab}	11.85^{ab}	60.6^{abc}	28.6^{ab}	11.2^{a}	228.04^{abcd}	181.89^{abcd}	409.92^{abcd}
6	PNM 50, RSM 50	134.6^{bcd}	91.7^{abc}	4.35^{c}	46.7^{cd}	32.9^{ab}	12.66^{b}	63.7^{a}	25.1^{ab}	11.0^{a}	215.32^{bcd}	141.35^{cd}	356.67^{cd}
7	PNM 25, RSM 75	134.8^{bcd}	91.4^{abc}	4.31^{c}	48.9^{bcd}	33.8^{ab}	12.04^{ab}	61.4^{abc}	27.9^{ab}	10.5^{ab}	213.16^{bcd}	160.33^{bcd}	373.49^{bcd}
8	RSM 100	125.8^{d}	89.8^{c}	4.33^{c}	45.3^{d}	33.2^{ab}	12.11^{ab}	63.4^{ab}	25.2^{ab}	11.2^{a}	204.65^{d}	133.92^{d}	338.57^{d}
9	PNM 50, SBM 50 + lysine	161.6^{ab}	92.5^{ab}	3.93^{abc}	59.2^{ab}	34.4^{ab}	10.92^{ab}	60.6^{abc}	28.9^{ab}	10.6^{ab}	247.90^{abc}	199.82^{ab}	447.72^{ab}
10	RSM 100 + lysine	131.1^{cd}	90.3^{bc}	4.24^{bc}	46.1^{cd}	32.4^{b}	12.71^{b}	63.8^{a}	24.7^{b}	10.9^{ab}	209.10^{cd}	140.53^{cd}	349.63^{d}
Sex													
	Male	188.7**	91.8	3.48**	69.8**	33.9	9.4**	61.2	28.6*	9.9**	280.97**	220.30**	501.27**
	Female	111.3	91.5	4.66	39.0	33.8	13.4	61.5	26.7	11.6	184.67	134.65	319.32
Grand mean		150.0	91.6	4.07	54.4	33.8	11.42	61.4	27.6	10.8	232.87	177.48	410.29
Standard error of mean		6.7	0.5	0.11	2.6	0.4	0.51	1.1	1.2	0.2	8.94	11.78	16.90

[1] Abbreviations: EBW = Empty body weight; LW = Liveweight; EFC = Efficiency of food conversion (g food/g EBW or DM); DM = Dry matter.

+ lysine) and 10 (RSM 100 + lysine) did not significantly alter these parameters compared with Diets 3 (PNM 100), 2 (PNM 50, SBM 50) and 8 (RSM 100) respectively.

Dry matter (DM) gain, DM percentage and DM EFC.

As with EBW traits, statistical similarity and overlapping between different groups were observed in DM gain, DM expressed as a percentage of EBW and in DM EFC. In general, the DM traits were in close agreement with the LW gains and lysine supplementation did not significantly modify them.

Protein, fat, ash and energy storage in the carcasses.

The pattern of similarities between treatments for protein, fat, ash and energy storage in the carcasses was as those already noted in the other carcass traits, but the relative superiority of the protein supplements was somewhat reversed, although average differences were not significant. For example, the average carcass protein percentages of 58.8 in Diet 1 (SBM 100), 58.4 in Diet 3 (PNM 100) and 63.4 in Diet 8 (RSM 100) were not significantly different, although these data suggest that rats fed RSM-supplemented diets tended to be slightly leaner. Using a SBM and fishmeal-supplemented basal diet, Bowland and Standish (1966) reported that for the period 2 to 6 weeks on test, protein retention, either on the basis of total protein consumed or on the basis of protein digested was consistently higher for rats receiving RSM than those on the basal diet. Although in the present study the differences in carcass leaness were not statistically significant, they may in fact be genuine dietary differences attributable to the relatively superior

N retention of rats fed RSM-supplemented diets.

Consistent with the higher protein percentages compared with fat in all carcasses, more energy was stored in the form of carcass proteins. However, due to the higher energetic value of fat, the difference in the amount of energy stored as carcass protein compared with that stored as carcass fat was not as large as the observed relative differences in carcass protein and carcass fat percentages. In a previous study (Orok and Bowland, 1973, unpublished data) in which Nigerian PNM was compared with SBM as protein supplement with Sprague-Dawley rats as the test species, a significantly ($P \leq 0.01$) higher carcass fat deposition was observed in rats fed Nigerian PNM-supplemented diets. In the present study, supplementation with US PNM did not produce fatter carcasses compared with rats fed diets supplemented with SBM or RSM. Rats fed RSM-supplemented diets tended to store less energy in their carcasses. This was probably primarily a reflection of their comparatively lower LW gain.

Sex had no significant effect on EBW, DM, and carcass protein percentages. In all other carcass traits, apart from ash percentage in which females were higher ($P \leq 0.01$) than males, the latter were superior ($P \leq 0.01$) to females. Age, breed, sex, species, plane of nutrition and physical environment affect body composition. Although there are species differences, males usually have less adipose tissue than females (Hafez, 1969). Because the rats, 9 weeks old at the termination of the study, were still relatively young, it is probably not surprising that EBW expressed as a percentage of LW showed no significant sex difference. At a later age when the reproductive organs and associated hormones are fully developed, there might be a larger sex difference in this trait.

The absence of a significant sex difference in the DM to EBW percentage appears to reflect the fact that the water content of the body is influenced more by age and body fat than by sex or body size (Roubicek, 1969); although it has been suggested (Weil, Jr. and Wallace, as reviewed by Speckmann *et al*., 1967) that "in the rat, with the possible exception of fat, the homeostatic mechanisms for maintaining the composition of the rat body operate within extremely narrow limits that have weight as a primary parameter and age as a minor secondary one." At various stages of growth, male and female rats have different compliments of hormones. To the extent that there is a marked, though not fully understood endocrine interrelationship with bone growth and maturation (Zobrisky, 1969), the observed significant difference ($P \leq 0.01$) in carcass percentage between male and female rats is not surprising.

OVERALL SUMMARY AND IMPLICATIONS FOR FURTHER STUDIES.

Meal by-products of oilseeds, of which PNM, SBM and RSM are examples, have individually faced some "ups" and downs" in world trade due to several factors, among which are their relative intrinsic values as protein supplements. There is no fixed status quo in the world market. Therefore, with continued technological progress and changes in human wants, none of these protein supplements can, in the face of a growing competition, be certain of a guaranteed relative importance in the world market.

This study was undertaken in the premise that effective research will not only reveal the relative worth of PNM, SBM and RSM as protein supplements, but will also indicate areas where improvements in the quality of each of these supplements can be effected to the benefit of producers and consumers. With rats as test species, PNM was as good a protein supplement as SBM while RSM was poorer. With pigs, the rank order of these supplements was SBM, RSM and PNM. These studies with pigs and rats as test species suggested major differences between the pig and the rat in lysine requirements. Lysine addition to the PNM-supplemented diet did not enhance the performance of rats; but lysine supplement increased, non-significantly, the feed intake of pigs with a concomitant improvement in ADG and EFC.

Rats responded positively ($P \leq 0.01$) to the addition of synthetic lysine to the RSM-supplemented diet. Postprandial lysine concentrations of pigs suggested that in RSM-supplemented diets, the metabolically useful lysine was low compared with the amount revealed by amino acid assay of the diet. Because the postprandial plasma lysine peak of pigs fed RSM-supplemented diets appeared relatively early and was high, and based on the literature evidence that transient storage of lysine in the muscle does occur, it is postulated that transient storage of lysine in the muscles of pigs fed RSM-supplemented diets is probably relatively inhibited.

Plasma urea proved to be a valid criterion of protein evaluation, when comparisons were based on the 0 - 7 h percentage increase in postprandial urea output/g N ingested on the morning of blood sampling, with correction for the initial (0 h) urea concentrations.

ADG was somewhat lower and the days to market longer for Pietrain-sired pigs than for crossbred counterparts; but the differences were not significant. Although the pigs were fed a finishing diet different from the starting diet whereas the rats were fed the starting diets throughout, it is noteworthy that in carcass composition, male rats had leaner ($P \leq 0.01$) carcass and better ($P \leq 0.01$) EFC than female rats. There was no significant sex difference in dressing (EBW/LW) percentage of male and female rats. Barrows were fatter ($P \leq 0.05$) and slightly, though not significantly, poorer feed converters than gilts. As with rats, there was no significant sex difference in dressing percentage. It is concluded that the various evaluation criteria employed in these studies may be useful in assessing protein supplements, especially if used in conjunction with each other and with other possible procedures.

Suggestions on future related studies seem appropriate at this stage. Just as breeding and selection has led to the production of high lysine corn and low erucic acid rapeseed, it seems within the realm of plant breeders to develop high lysine peanuts. Secondly, the optimum dietary DE and DE-amino acid ratios to improve the carcass grade index of pigs fed PNM-supplemented finishing rations seems to be an important consideration for the practical nutritionist to study. Thirdly, it is evident from the comparatively severe goitrogenecity of Span RSM that both the plant breeder and the processor still have unresolved tasks. However, development of low glucosinolate rapeseed is at an advanced stage, and should eliminate this problem. Fourthly, it would be interesting to ascertain, if the *in vitro* enzymatic degradation of lysine is stimulated by RSM extracts. Such a study might be extended to ascertain the extent of storage of free ^{14}C-lysine and protein bound ^{14}C-lysine in the muscle of starting pigs fed the various diets used in this study.

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APPENDIX i

Column specifications for amino acid analysis of feeds and blood plasma: Type JLC - 5AH amino acid analyzer.

FEEDS

Column length

Short column (for basic amino acids): 15 cm.

Long column (for acidic and neutral amino acids): 55 cm.

Resin

Short column: AR 15[1], particle size 16 - 17 microns; divinyl benzene (DVB) cross linkage 8.5%.

Long column: AR 50[2], particle size 18 - 20 microns; DVB cross linkage 8.5%.

Buffer system

Short column: Sodium citrate buffer pH 5.28 throughout the analyses.

Long column: Sodium citrate buffer pH 3.27 for 125 min.; pH 4.25 thereafter.

Pump flow rates

Buffer pump: 0.8 ml per min.

Detection pumps (a) Sampling pump: 0.42 ml per min.

(b) Ninhydrin pump: 0.21 ml per min.

[1, 2] Jealco (U.S.A.) Inc., 477 Riverside Avenue, Melford, Mass. 02155, U.S.A.

Temperatures

Reaction bath: 95 C

Short and long columns: 55 C

Blood plasma (a physiological fluid)

Column length and Resin: As above, except that for the long column, the particle size of the resin was 12 ± 2 microns; DVB cross linkage 9%.

Buffer system

Short column: Sodium citrate buffer pH 4.15 for 99 min.; then pH 5.36 for 72 min.

Long column: Lithium citrate buffer pH 2.62 for 120 min.; then pH 3.00 for 120 min.; then pH 3.90 for 150 min.

Pump flow rate: As above.

Temperatures

Reaction bath: 95 C

Short column: 45 C

Long column: 45 C for 150 min., 55 C thereafter.

APPENDIX TABLE 1A: POSTPRANDIAL AMINO ACID PATTERNS (µm %)**

Diet number*	1					2					4					7				
Supplemental protein source(s) % PNM	---					50					100					25				
RSM	---					---					+					75				
SBM	100					50					lysine					---				
Postprandial interval h	0+	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Essential amino acids																				
Arginine	10.3	22.7	18.7	21.0	17.7	8.3	32.6	25.1	25.0	25.4	15.4	38.1	36.5	30.2	28.1	20.5	35.9	25.9	22.0	18.0
Histidine	7.0	12.2	11.8	12.4	10.1	7.6	13.8	12.0	11.9	12.0	9.1	12.4	11.9	11.6	11.4	11.9	18.1	15.5	14.9	14.0
Isoleucine	14.0	17.5	16.2	16.0	14.0	10.9	18.6	15.7	15.1	16.1	11.5	16.0	15.1	13.3	13.9	15.0	20.3	15.4	13.1	13.0
Leucine	21.8	25.2	22.4	21.6	18.2	14.5	25.5	20.3	19.6	20.5	13.6	20.6	20.4	17.9	18.5	18.9	27.5	20.5	17.1	16.7
Lysine	17.2	30.0	21.1	22.9	17.0	12.1	24.1	14.7	13.4	12.0	18.3	27.4	22.0	15.6	12.4	23.2	35.0	20.8	14.8	11.0
Methionine	3.0	4.8	4.6	4.5	4.1	3.0	5.7	4.7	4.4	4.8	4.3	5.9	6.0	4.9	5.0	5.1	8.0	6.7	5.5	5.2
Phenylalanine	8.2	13.6	13.9	13.5	11.5	7.4	17.0	17.1	15.8	15.0	8.4	15.4	15.8	13.9	13.8	10.1	16.6	14.0	12.1	11.3
Threonine	13.2	21.4	19.1	19.2	17.9	11.5	20.4	17.3	16.6	17.1	14.8	19.6	17.5	14.6	14.1	14.9	25.2	19.7	16.5	15.4
Tryptophan[1]	4.5	6.7	6.1	6.0	6.3	4.1	8.4	6.6	6.0	6.6	4.4	6.6	5.9	5.5	5.7	4.4	7.9	6.4	5.7	5.2
Valine	35.6	42.0	37.8	36.6	32.9	24.1	39.3	34.6	33.1	34.2	22.9	32.5	33.2	30.8	30.9	31.4	44.0	35.7	32.0	32.1
Non-essential amino acids																				
A-amino-N-butyric acid	2.1	1.9	1.4	1.2	1.2	1.4	1.4	1.1	0.9	1.1	1.5	1.2	1.0	0.9	0.8	1.4	1.8	1.7	1.6	1.5
Alanine	38.9	72.1	63.9	58.8	55.4	42.4	85.6	75.8	71.2	73.9	60.4	99.8	100.2	78.8	79.8	72.0	112.4	92.8	81.7	81.7
Ammonia	66.4	65.5	52.1	59.5	59.2	56.4	59.6	57.7	55.7	62.0	66.6	59.0	61.6	59.9	58.1	76.1	86.1	75.8	70.8	70.4
Asparagine	3.6	8.4	8.0	8.1	7.5	5.1	12.4	11.1	10.5	11.1	7.0	12.6	13.6	10.6	10.9	8.4	14.4	10.6	8.3	8.1
Aspartic acid[2]	2.0	2.8	1.8	2.0	2.6	2.0	2.9	2.6	2.7	2.7	2.4	2.9	2.9	2.2	1.6	2.6	4.2	2.8	2.4	1.5
Cystine[3]	11.1	12.0	10.7	10.9	11.0	9.9	11.7	10.6	9.8	11.4	8.1	9.7	11.0	9.4	11.1	8.7	12.7	10.8	9.4	10.4
Glutamic acid	18.6	23.5	16.8	16.4	16.3	17.3	23.6	21.9	20.7	19.2	20.4	21.5	20.5	16.2	17.4	25.3	37.7	29.6	24.6	23.1
Glycine	83.2	87.1	75.2	76.6	79.9	91.9	102.7	90.1	91.4	94.0	118.9	124.1	126.7	108.7	111.0	101.1	124.3	104.2	101.2	99.4
Hydroxyproline[2]	7.2	8.7	7.9	7.7	10.8	8.6	9.9	8.0	7.6	9.6	11.5	10.8	9.8	8.4	9.3	11.2	10.4	8.4	8.5	8.0
Ornithine	5.6	10.9	10.7	10.8	9.1	6.1	15.7	14.8	14.2	14.2	6.8	15.9	17.8	16.7	16.4	9.7	18.8	16.6	15.4	13.3
Proline	16.1	47.1	48.3	49.0	44.4	20.5	65.0	62.8	60.3	64.8	26.6	62.5	70.3	63.7	68.8	35.5	69.2	70.5	66.8	60.8
Serine	13.1	19.3	17.8	18.1	16.9	15.4	25.4	22.7	23.5	23.7	22.2	29.1	29.1	25.4	27.0	18.5	27.2	20.6	18.0	17.2
Taurine	17.4	23.9	20.2	18.7	17.1	17.0	22.7	19.2	17.7	15.8	14.8	18.6	16.5	13.9	13.6	17.7	28.0	19.9	17.8	17.3
Tyrosine	6.9	13.0	14.3	13.4	11.5	7.6	18.0	18.4	17.3	17.6	8.9	18.5	20.2	18.7	18.4	9.5	16.6	15.0	12.8	11.8

** Values are means of 2 Replicates based on amino acid analyses of pooled samples: 5 ml from each donor, 3 donors in Replicate 1, 4 donors in replicate 2.

* Diets are arranged in descending order of nutritiveness as determined by gain and/or increase in plasma urea output/N ingested %.

+ Postprandial interval "0" is just before re-feeding.

[1] Tryptophan aminogram frequently had 2 peaks, suggestive of interference from unidentified amino acid or other substances.

[2] Optimum pH for satisfactory separation of aspartic acid and hydroxyproline was 2.5. At pH 2.0 to 2.2 encountered in a few analyses in Replicate 1, aspartic acid and hydroxyproline interfered with each other.

[3] Unsymmetrical cystine aminograms were obtained in a few instances in Replicate 1, suggestive of slightly elevated values.

APPENDIX TABLE 18: POSTPRANDIAL AMINO ACID PATTERNS (μm %) (Continued)

Diet number			8					6					5.					3		
Supplemental protein source(s)% PNM			---					50					75					100		
RSM			100					50					25					---		
SBM			---					---					---					---		
Postprandial interval h	0	1	2	3	4	0	1	2	3	4	0	1	2	3.	4	0	1	2	3	4
Essential amino acids																				
Arginine	18.6	23.7	17.8	18.6	15.2	19.2	35.3	28.5	25.2	19.3	18.3	36.5	24.8	24.8	19.1	18.5	33.6	25.1	23.6	18.9
Histidine	13.3	17.3	15.7	15.5	13.9	11.2	15.3	13.9	13.5	12.2	10.2	14.9	12.8	13.1	11.6	9.4	11.3	10.7	10.8	9.9
Isoleucine	14.9	17.6	12.8	12.5	11.8	13.7	19.3	15.5	14.7	13.8	12.7	17.7	14.1	15.5	14.0	12.1	15.8	13.3	13.3	12.9
Leucine	20.4	24.5	18.2	17.8	16.4	15.2	24.8	19.7	19.0	17.5	14.6	23.1	18.4	20.0	17.8	12.7	19.6	17.1	17.0	16.0
Lysine	28.8	24.7	24.0	19.9	16.2	19.0	25.1	14.7	10.4	7.9	13.9	17.1	7.9	6.5	5.8	11.8	11.4	6.5	6.2	6.1
Methionine	5.5	7.5	6.0	6.0	5.4	4.9	7.5	5.9	5.5	5.0	4.5	6.6	5.6	5.4	4.8	4.2	5.4	5.1	4.8	4.4
Phenylalanine	9.5	13.2	11.0	10.8	10.0	8.5	15.8	14.1	13.5	12.1	9.8	17.3	15.2	16.1	13.4	10.1	16.5	15.4	15.4	14.1
Threonine	15.3	20.1	14.6	15.5	14.3	17.7	27.2	22.9	21.2	19.1	23.6	29.0	22.8	23.8	21.9	17.5	20.2	17.8	17.0	17.0
Tryptophan	4.0	6.8	5.7	5.4	5.0	4.5	7.0	5.4	5.0	4.8	4.0	6.9	4.9	5.1	5.0	4.4	6.1	5.2	5.4	6.0
Valine	35.6	40.6	33.1	32.4	30.9	25.8	37.5	33.7	32.6	31.4	25.1	35.3	30.5	33.9	31.5	21.7	30.2	27.9	29.1	27.7
Non-essential amino acids																				
A-amino-N-butyric acid	1.8	1.9	1.7	1.6	1.7	1.3	1.6	1.4	1.8	1.4	1.1	1.1	1.0	1.1	1.0	0.7	0.7	0.8	0.8	0.7
Alanine	69.1	92.8	76.6	70.2	66.9	75.7	115.7	105.0	95.3	85.9	82.5	124.2	104.8	98.2	94.6	83.2	111.4	100.8	96.7	95.6
Ammonia	82.7	81.3	81.5	80.8	72.0	74.5	74.1	62.6	68.2	70.9	67.7	72.5	63.8	62.9	63.9	61.7	64.7	56.4	59.8	58.5
Asparagine	7.1	9.6	6.2	6.1	6.5	8.7	15.2	12.4	11.1	9.6	10.3	16.2	12.4	11.6	9.8	8.9	13.2	11.3	10.0	9.0
Aspartic acid	2.6	3.2	2.6	2.0	1.8	3.1	3.2	3.0	2.3	2.2	2.2	2.9	2.9	3.0	1.9	2.2	2.6	2.1	2.0	2.7
Cystine	10.9	12.4	10.9	9.3	10.1	9.4	11.8	11.0	10.4	11.6	7.6	10.0	9.6	8.7	11.7	7.1	8.8	9.0	8.8	11.4
Glutamic acid	26.3	34.3	27.5	29.1	23.8	27.9	33.7	30.6	26.3	25.0	23.7	29.5	23.8	24.2	22.9	23.9	25.9	19.6	21.1	21.0
Glycine	111.2	110.0	97.8	96.7	94.2	112.2	122.8	110.2	108.6	100.4	120.0	131.2	114.1	116.9	105.6	115.4	123.9	110.8	109.5	107.6
Hydroxyproline	10.0	9.6	7.9	8.1	7.8	10.2	8.8	7.0	7.4	6.9	10.0	9.4	8.2	7.2	6.8	10.1	8.1	7.3	9.4	7.6
Ornithine	9.9	14.9	14.1	13.0	11.2	9.7	18.0	19.2	18.6	16.1	8.9	18.0	17.1	18.0	15.9	8.1	16.4	16.4	18.9	16.6
Proline	41.1	55.2	55.3	53.3	46.7	35.3	76.6	70.0	69.3	57.5	35.7	74.1	66.2	64.1	56.5	31.4	59.9	59.1	56.4	58.6
Serine	17.8	21.7	16.6	16.8	16.4	22.4	31.2	26.8	25.8	23.3	27.6	34.8	29.1	31.5	28.6	27.2	32.8	30.1	30.4	29.1
Taurine	17.0	20.9	19.0	16.2	15.9	18.0	18.3	16.5	15.7	14.0	15.7	18.9	16.3	14.5	14.2	15.4	18.1	13.7	13.7	14.6
Tyrosine	8.6	12.8	10.7	10.0	9.0	8.1	15.5	14.8	13.9	12.6	9.5	17.9	17.2	16.9	15.5	10.5	18.2	18.0	18.4	16.8

APPENDIX TABLE 2A: FATTY ACID PERCENTAGES OF DIETARY LIPIDS (BY ANALYSIS)

		Starting Diets					
Diet numbers		1	2	3	4	5	6
Supplemental protein source(s) %	PNM	---	50	100	100	75	50
	SBM	100	50	---	+	---	---
	RSM	---	---	---	lysine	25	50
Lipid content of diets %		4.0	4.2	4.8	4.8	4.5	4.4
Saturated fatty acids		35.3	32.6	29.9	30.0	30.3	33.1
Unsaturated fatty acids							
(a) Mono-unsaturated fatty acids		33.0	34.3	38.0	38.0	38.2	39.1
Erucic acid		----	----	----	----	0.2	0.5
(b) Poly-unsaturated fatty acids		31.7	33.1	32.1	32.0	31.5	27.8
		100.0	100.0	100.0	100.0	100.0	100.0

APPENDIX TABLE 2B: FATTY ACID PERCENTAGES OF DIETARY LIPIDS (BY ANALYSIS)

		Starting Diets				Finishing Diets[+]	
Diet numbers		7	8	9	10	11	12
Supplemental protein source(s) %	PNM	25	---	50	---		
	SBM	---	---	50	---	SBM	PNM
	RSM	75	100	+	100+		
				lysine	lysine		
Lipid content of diets %		4.9	4.3	4.1	4.5	1.4	1.9
Saturated fatty acids		34.0	32.6	32.1	33.3	32.4	33.3
Unsaturated fatty acids							
(a) Mono-unsaturated fatty acids		40.1	41.0	35.2	40.9	35.3	40.4
Erucic acid		0.8	1.0	----	1.0	----	----
(b) Poly-unsaturated fatty acids		25.9	26.4	32.7	25.8	32.3	26.3
		100.0	100.0	100.0	100.0	100.0	100.0

[+] SBM = soybean meal-supplemented finishing diet; PNM = peanut meal-supplemented finishing diet.